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The Problem of Osteoporosis

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Osteoporosis is a nonspecific endocrine disorder resulting from at least 12 different causes and many combinations of causes and characterized by loss of internal lamellae of cortical bone and a diminution in the number of trabeculae of cancellous bone. The pathologic picture is highly stereotyped and suggests that there is one mechanism common to all causes, although these are as diverse as pregnancy, castration, starvation, diabetes mellitus, prolonged calcium deprivation, Cushing's syndrome, acromegaly, disuse atrophy, total immobilization of paraplegics, multiple traumata and the post-traumatic adaptation syndrome, idiopathic hypoparathyroidism, rheumatoid arthritis and other conditions of unknown etiology. It is possible that this one mechanism involves the effects of an adrenal cortical steroid hormone or one of its metabolites upon bone tissue. Osteoporosis, regardless of the clinical circumstances in which it is found, may be a fractional form of Cushing's syndrome, a form that appears typically in middle-aged individuals. Accordingly, osteoporosis as it is most commonly seen in women after natural or artificial menopause, like Cushing's syndrome, may be produced by the glucocorticoid hormones causing cessation of osteogenesis and resorption of bone. Furthermore, it is possible that the beneficial effects of gonadal hormones upon patients with osteoporosis are not due to any direct action of estrogen or androgen upon bone tissue but to an effect in suppressing the secretion of corticoid hormones by the adrenal cortex. Both clinical and experimental support can be found for these statements in the literature on osteoporosis and in recent observations that are presented here in preliminary form.

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The extensive reviews in the recent literature[1-7] on clinical osteoporosis all emphasize the significance of the outstanding contribution of Albright and his associates.[8] Reifenstein and Albright[9] defined osteoporosis in their definitive report in 1947 as a condition resulting from "too little bone formation" on the basis of metabolic balance studies on five postmenopausal women, three women with Cushing's disease, one senile man, one woman immobilized by multiple fractures and one woman paralyzed and immobilized by poliomyelitis. Since then, similar investigations of many more patients confined to metabolic wards corroborated their discovery that sex hormones can in some unknown way modify the progress of the disease. In 1948, Albright and Reifenstein advanced the hypothesis that estrogen deficiency was the cause of osteoporosis. In 1957, Reifenstein[6,7] modified this position to include the possibility that senile osteoporosis and other forms were caused by a state of imbalance between anabolic or gonadal hormones and antianabolic or adrenal corticoid hormones. The concept of antianabolism was employed in contradistinction to catabolism to propose that osteoporosis developed as a result of inhibition of synthesis of the protein of the bone matrix. The reasoning behind this interpretation was based on metabolic balance studies on patients with Cushing's syndrome.[10] Experimental investigations, however, on animals treated with cortisone, revealing retention of sodium and excretion of large amounts of potassium (liberated by breakdown of cytoplasm of cells), suggest that catabolism is the chief action of glucocorticoid hormones.[11,12] It is unfortunate that the terms anabolism, antianabolism and catabolism have such broad meaning that they obscure the fact that metabolic process may proceed in different directions all at once in a complex tissue such as bone. With our present, scant knowledge of the body chemistry of protein it is doubtful that these terms contribute very much to further our understanding of atrophy or osteoporosis. Catabolism

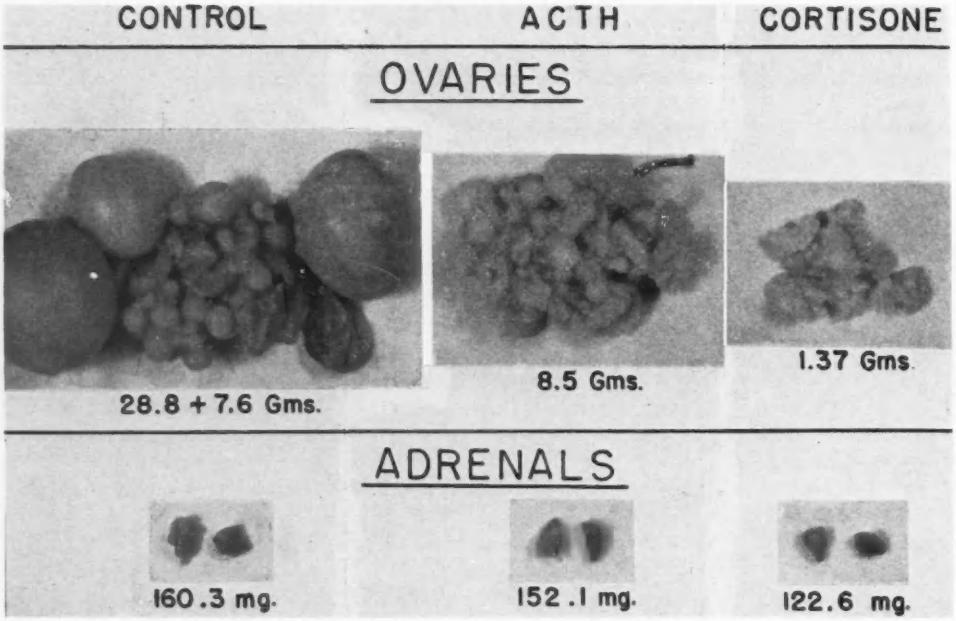

FIG. 1.—Photograph showing the size and weight of a typical series of right ovaries and right and left adrenal glands in a control laying hen, a hen treated with 120 units of ACTH (corticotrophin) per week for 4 weeks and a hen treated with 300 mg. of cortisone per week for 4 weeks. The number of ova in the ovary appears to be the same in the untreated and treated birds. The hen treated with ACTH, however, shows only small deposits of egg yolk in a few of the follicles. The hen treated with cortisone shows absolutely no synthesis and storage of egg yolk. The adrenal glands show atrophy only in the cortisone-treated bird. Egg production ceased in the birds treated with ACTH or cortisone. Their long bones showed enlarged vascular spaces and osteoporosis of the cortex, while their marrow cavities became filled with endosteal new bone.

refers to a specific series of chemical reactions that may lead to the process of bone resorption and eventually result in osteoporosis.

The concepts of *labile* and *stable* protein have been used to distinguish chemical from morphologic aspects of metabolism. The labile protein, by definition, is that fraction of the mass of the total protein in the body that is dispensable, as, for example, protein catabolized or excreted as nitrogen following fasting and mobilized without morphologic change in tissue. The stable protein, by definition, is that fraction of the body protein mass that is not labile and is closely related to such morphologic structures as collagen and bone matrix. So little is known about the turnover of the stable fraction of the body protein that it is not possible to correlate the results of balance studies with changes in bone tissue. It is clear, however, that 35 per cent of the fat-free dry weight of bone tissue is densely packed, pure collagen, and that one-third of this large mass of collagen can disappear from the skeleton of the average patient with osteoporosis before the condition is detectable by x-ray examination.

Experimental Osteoporosis: A disorder of the skeleton resembling clinical osteoporosis has been produced in birds[13] and rabbits[14] injected with large doses of ACTH or cortisone. Young, rapidly growing mice, guinea pigs and rabbits, and rats of all ages, appeared to be resistant to the action of cortisone on bone. It is not generally appreciated, however, that experimental and clinical osteoporosis characteristically occur in adult or aged individuals and can be detected in the early stages only in preparations of cross-sections of cortical bone. A limited form of osteoporosis has been observed also in rats reared on calcium-deficient diets[15] during repair of multiple fractures[16] and during inanition.[13] Osteoporosis due to starvation is often described in the clinical literature as

hunger osteopathy, but like all other forms of osteoporosis this also seems to be associated with the action of adrenal cortical hormones. Fasting human subjects excrete larger quantities of 11-oxycorticosteroids.[17] Starved guinea pigs show hypertrophy of the zona fasculata which secretes 11-oxycorticosteroids and is under the control of ACTH.[18] Birds with osteoporosis produced by injections of cortisone develop hypercalcemia and enlarged vascular channels in the cortex within a few weeks to indicate that bone resorption plays a part.[13] Histologically, the osteoporosis produced by cortisone in experimental animals is indistinguishable from osteoporosis seen in Cushing's disease, castration or spontaneous menopause, or senility in patients. Birds with hypercortisonism due to exogenous cortisone or prednisone and osteoporosis, treated with sex hormones, also showed nonspecific beneficial effects like those seen in patients with osteoporosis treated with estrogen. The treatment reduced the rate of weight loss and the mortality but did not reverse the osteoporosis. The osteoporosis, of course, gradually disappeared and the bone density was restored when cortisone was discontinued. If the cause of osteoporosis were similarly removed in patients with so-called postmenopausal osteoporosis, it is reasonable to expect that bone density would be restored in 10 years, if not sooner. It is not reasonable to assume that it should require as long to produce a cure as the length of time that the disorder was present prior to the onset of treatment; this is certainly not the case in scurvy, osteomalacia or other diseases about which knowledge of etiology is more adequate.

Osteoporosis occurs in nature in animals, presumably as a manifestation of the action of corticotrophin. In birds, particularly those that fly north to reproduce where the food supply is limited, the females experience a menopause-like state every year and develop osteoporosis and even spontaneous fractures.[19] Large amounts of bone are removed from the intramedullary deposits to mobilize calcium to transport protein during growth of the ovum and to calcify the egg shells.[13] After the eggs are hatched and the young are reared, the birds molt. To mobilize protein, bone tissue is again absorbed, but this time the compact bone resources are tapped not for a supply of calcium but for a supply of protein and amino acids needed for the growth of new feathers. The results are osteoporosis, loss of mechanical support and the spontaneous fractures. The same type of bone resorption and osteoporosis occurs in deer[20] growing new antlers during the period in the early spring when the diet is low in protein. Similar effects were produced experimentally in capons, hens and roosters treated with large doses of ACTH or cortisone.[13] The response of the hen was cessation of egg-laying within one week, atrophy of the ovary within three weeks and osteoporosis after eight weeks (fig. 1). The rarefying effects on the bones were the most severe of all in the capon. The rooster showed only slight changes in the bones within a period of eight weeks of cortisone treatment but eventually developed hypercalcemia and osteoporosis. The phenomenon of bone resorption appeared to be more prominent than inhibition of bone formation, inasmuch as endogenous estrogen as well as estrogen treatment produced intramedullary new bone in large quantities in birds even with very severe experimental Cushing's syndrome. Estrogen treatment may inhibit bone resorption through an inhibitory effect on either the anterior pituitary secretion of corticotrophin or the adrenal secretion of corticoid hormones.

It is important to note that the experimental basis of the treatment of osteoporosis with sex hormones came not from treatment of animals with experimental osteoporosis but from the treatment of normal animals with normal skeletal tissues.[21-25] This produced a response described in birds and mice as intramedullary bone formation and in growing rats as accumulation of spongy bone due to inhibition of bone resorption. These responses have not been found in other mammals, including man, except insofar as estrogen inhibits growth and bone resorption in growing individuals and perhaps only bone resorption in mature individuals. Duckworth and Ellinger[26] depleted the skeletons of rats with calcium-deficient diets and lactation, then removed the ovaries. There was no difference in the rate of replacement of the bone tissue in untreated rats compared with rats treated with estrogen, suggesting that factors other than estrogen were responsible for repair.

From all of the various natural and laboratory reactions described above, it is apparent that the adrenal corticoid and gonadal glands and hormones interplay in a definite way to affect the structure of bone, and that osteoporosis may be a disturbance resulting from an excess of endogenous hydrocortisone. The literature on the adrenogonadal interaction (or mechanism)

was reviewed by Burrows in 1949, Engel in 1953 and Jones[12] in 1957. In young growing animals, it is greatly modified if not controlled by the hypophysis. After growth is finished, and especially in aged animals at the conclusion of reproductive functions, there is a lessening in the potency of the hypophyseal-gonadal mechanism and release of corticoid hormones from the restraining action of gonadal hormones. For example, in young birds or rats, estrogen stimulated secretion of ACTH and corticoid hormones and suppressed growth. In mature rats, large doses of estrogen or castration produced degeneration of the fasciculate zone of the adrenal cortex. In patients with Cushing's syndrome, estrogen reduced nitrogen and potassium excretion and presumably inhibited the secretion and overaction of glucocortoids on protein stored in bone and other tissues of the body.

Pathogenesis: The outstanding pathologic changes that lead to osteoporosis are: (1) modulation of osteocytes and osteoblasts and (2) incomplete or suspended remodeling of cortical bone. Modulation is simply reversion of large proliferating (polygonal) osteoblasts to small connective tissue cells (spindle-shaped) or resting forms; this change is reversible in response to injury, as can be seen in rapid healing of fractures in osteoporotic bone. Modulation occurs in the bones of women after the menopause, in aged individuals, in fasting subjects or in patients with hyperthyroidism or diabetes and other such diverse conditions, and is closely associated with the disappearance of both cancellous bone and cortical bone. But the loss of cortical bone is most important since it brings about the symptom of pain and the spontaneous fractures of osteoporotic bones.

The compact or cortical bone tissue is composed of haversian systems or osteones and is metabolically much more active than was ever suspected before the recent development of biophysical methods for study of bone tissue.[29] Remodeling of cortical bones, as can be seen only by "triple preparation" of a single section by microradiography, microautoradiography and histochemistry, consists of two phases: resorption and reconstruction of lamellar bone. These two processes are concerned with the normal daily turnover of calcium, phosphorus and nitrogen and are continuously active even in senescent, aged individuals; indeed, in response to prednisone treatment, the resorption phase can become so accelerated as to destroy 30 per cent of the bone mass in an adult patient in less than 9 months. From all present knowledge of the physiology of bone, it is reasonably certain that haversian remodeling is not under the control of the parathyroid hormone. The evidence, as it arises from observations in birds,[13] suggests that hydrocortisone has a specific role in haversian remodeling, and that the osteoporosis is essentially a disturbance of the processes concerned with this phenomenon. In osteoporosis, there is tunneling of wide channels through old osteones by new blood vessels, and the first or resorption phase is thereby accelerated; in cortisone-treated birds, the bone replacement phase, to substitute the old osteones for new ones, is slowed, then suppressed, and later omitted entirely.

The pathologic picture in osteoporosis does not include osteoclasts. These cells are regarded as neither increased or decreased in number, but actually it is difficult to demonstrate osteoclasts in adult individuals, and it should be recognized that bone resorption can occur without the formation of these cells. In osteoporosis, bone resorption occurs without formation of osteoclasts, and in this way it is like bone resorption following vitamin A poisoning, or inanition, but differs from bone resorption in chronic hyperparathyroidism. Bone resorption occurs in hyperparathyroidism to mobilize calcium stored in the skeleton. The stimulus that normally activates the parathyroid glands to secrete parathyroid hormone to produce osteoclasts and resorb bone is a fall in the level of calcium ion in the blood plasma.[22] The stimulus that activates the pituitary to produce corticotrophin has been regarded as a neurohumoral influence from centers in the hypothalamus. The stimulus that affects the hypothalamus, whether it is the level of hydrocortisone itself or some metabolite of protein, is not known. In any case, the bone is apparently resorbed without any preceding change in the calcium ion concentration of the blood and presumably by some stimulus to mobilize protein rather than calcium from bone matrix.

Bone resorption is either normal or osteoporotic bone always occurs with removal of the matrix and the mineral concomitantly. The mobilization of the nitrogenous substances of matrix automatically transfers calcium and phosphorus from the bone salt to the extracellular fluid, blood plasma and urine. A *negative calcium balance* can be observed in patients with osteoporosis by

metabolic balance studies if the amount of calcium excreted via the urine and feces exceeds the amount absorbed from the daily diet. The mobilization of and the excretion of nitrogenous degradation products of protein of bone matrix and excretion of amino acids not used for the synthesis of new bone (to replace bone lost by resorption) generally would be expected to appear as a *negative nitrogen balance*. If the loss of skeletal tissue were proportionately higher than the loss of soft tissue in the body in osteoporosis, there would be a priori more calcium lost than nitrogen. The nitrogen equivalent to the calcium loss is so small that it is difficult to find a negative nitrogen balance in patients with severe postmenopausal osteoporosis, and yet in these same individuals it is possible to produce a positive nitrogen volume after administration of testosterone. Possibly the nitrogenous substances mobilized from bone are retained elsewhere in the body. The relationship between nitrogen turnover in muscle and nitrogen turnover in bone is not understood and needs further study in patients with osteoporosis.

With the aid of biopsy specimens, it is generally not difficult to distinguish osteoporosis from osteitis fibrosa (hyperparathyroidism) in which proliferation of fibrous connective tissue cells is marked, or from osteomalacia in which there is failure of calcification with osteoid borders on the new bone trabeculae. Osteogenesis may not be as inhibited as it would appear in either Cushing's syndrome or postmenopausal osteoporosis or senile osteoporosis. Proliferating osteoblasts are normally scarce in adult bone, and the absence of these cells in osteoporosis is not surprising. Indeed, osteoblasts do appear and produce bone protein and new bone matrix in response to the stimulus of injury following fractures in osteoporotic bones just as in normal bones.

The very existence of osteoporosis is prima facie evidence of bone resorption, and therefore it remains to be proven that the rate of resorption is the same as in normal individuals. The mechanism of osteoporosis in Cushing's syndrome, explained by the so-called antianabolic effects of corticosteroids, is based on the assumption that bone resorption is not increased and that the loss of bone mass in osteoporosis occurs at the rate of activity of bone resorption in a normal adult individual. The morphologic picture showing neither osteoclasts nor osteoblasts could be interpreted as meaning that bone replacement is suppressed but not that bone resorption is normal, because bone can be resorbed rapidly without osteoclasts, while it cannot be formed without osteoblasts. At any rate, this morphologic picture may be produced as well by catabolism as by antianabolism. The results of studies on the effects of cortisone on all kinds of body organs and biologic systems show that it is not possible to distinguish between antianabolism and catabolism by net nitrogen change.[11,12] Lysis of tissue protein and other evidence of catabolism can be seen in the phosphaturic action of cortisone in rats, in liberation of kidney and hepatic arginase by cortisone and in mobilization of potassium from cells.[30,31] Radioactive isotopes, e.g., Ca^{45}, have been used in adrenalectomized rats, and Ca^{47} is now in use on patients with osteoporosis and provides a quantitative method that could prove or disprove the hypothesis that the hormones of the adrenal cortex accelerate resorption of bone in postmenopausal osteoporosis.[32]

Albright and his group[8] introduced the term antianabolism to correlate the lack of cellular proliferation with the metabolic changes responsible for diversion of amino acids and fatty acids to the formation of sugar and prevention of synthesis of new protein. Later, Reifenstein[10] recognized that when the amounts of amino acids and/or fatty acids that can be derived from antianabolism of protein are not adequate to meet the demands for anabolism of carbohydrate, the corticosteroids induce a catabolic effect upon protein and fat (in addition to the antianabolic effect). The mechanism appears to be the same as described in rats, first by Cuthbertson and later by Urist and McLean[16] who observed the postshock metabolic response in which the organism, presumably under the influence of ACTH, actually preferred to catabolize endogenous rather than exogenous protein to meet the exigencies of the moment. Astwood,[11] in a keynote address before a round-table discussion on energy metabolism, concluded that the major action of corticoids was lysis of tissue protein. This leads to excretion in the urine of intracellular electrolytes and the products of nuclear disintegration. Liberation of amino acids from the body comes nearer than any other to being a direct action of adrenocortical steroids. Houssay[11] postulated a permissive action of the adrenal corticoids on various endocrine reactions of the gonads and thyroid glands. Samuels[11] correctly criticized the present tendency of clinicians to use the term protein too

broadly, because cortisone could affect different proteins in different ways. Fasting rats and rats with multiple healing fractures show that building materials released by old cortical bone and muscle can be metabolized for more essential functions such as liver metabolism, fracture healing, etc., and supplied at the expense of the skeleton to result in osteoporosis.

While calcium metabolism is apparently not at fault, calcium and phosphorus intake and excretion are relatively easy to measure and should give a good indication of the loss of bone substance in patients with postmenopausal osteoporosis or Cushing's syndrome. Nitrogen intake and output are also measured at the same time, and they are actually related to the pathogenesis of osteoporosis more specifically than calcium. Nitrogen, however, has a more general distribution throughout the metabolic pool and involves every tissue and organ in the body, and its turnover is correspondingly very difficult to interpret in terms of bone. Nitrogen turnover should be measurable, nevertheless, with the aid of either stable or radioactive isotopes of nitrogen, because decalcified bone matrix contains a large concentration of protein stored as collagen. Crispell, Parson and Harden[33] measured the N^{15}-N^{14} ratio of urinary nitrogen by mass spectrometer analyses and found that nitrogen was not excreted in large amounts by patients with Cushing's syndrome, as it is by normal subjects, when they fed on high protein diets. These observations did not contribute information about bone as a target tissue and cannot be interpreted as though nitrogen retention was synonymous with bone formation. The blood plasma is supersaturated with calcium, phosphorus and carbonate in patients with osteoporosis just as it is in normal patients, and it can be safely assumed that if the bone matrix were replaced, there would automatically be deposition of bone salt in patients with this disorder. There is no cellular proliferation or bone deposition, however, in osteoporosis, but only deleterious bone resorption and an inexorable loss of calcium and phosphorus in the urine and feces; if antianabolism were the mechanism responsible at the beginning of this process, it would seem that it is most certainly resorption that produces the osteoporosis that is found at the end. To explain the antianabolic effects in one area and the catabolic effects in another area, Reifenstein postulated "local factors."[6,7]

Clinical Signs of Adrenal Hypercorticoidism in Senile Osteoporosis: The writer has served in the capacity of consultant to a sorority home for the aged for a period of approximately 10 years and has had the opportunity to observe 141 women with an average age of 85. The incidence of pain in the dorsal spine was 25 per cent. The incidence of fracture of the neck of the femur was 15 per cent. Nearly all of these fractures were associated with minimal trauma in which there was some question whether the fracture occurred first and the patient fell afterwards, or whether the fall occurred first and the fracture occurred as the result. In any case, the force of the trauma was very slight. Osteoporosis was more readily apparent in the axial skeleton and the superior portion of the neck of the femur where the bones have a thinner cortex than in the appendicular skeleton where the bones have a thick cortex. Bending of the vertebral end-plates and fractures occurred when the resorption of the lamellar bone caused collapse or disintegration. The loss of x-ray density was due chiefly to the loss of thickness of the cortex more than to the trabecular bone in the case of the neck of the femur. The wedge-shaped vertebrae in the dorsal spine and a tendency toward the development of biconcave vertebrae in the lumbar spine were dependable and objective evidence of osteoporosis. Radiolucence was largely a subjective observation, variable with radiologic technic and generally not reliable evidence of osteoporosis. In addition to gross deformities and fractures, 40 per cent of these aged women showed more than 5 of the following 11 signs ordinarily associated with Cushing's syndrome: (1) the "dowager's hump" in the cervicodorsal region of the spine; (2) one to three inches loss of height; (3) thin, reddened skin; (4) slight hirsutism; (5) slight striations of the abdominal skin; (6) enlargement of the clitoris; (7) low-grade diabetes; (8) hypertension; (9) obesity; (10) slight swelling of the ankles; (11) slight rounding of the face. That the process of aging thus often includes hormonal adrenogonadal imbalance and a limited form of Cushing's syndrome was suggested by Reifenstein.[3] These same signs can be found in younger patients with postmenopausal or other forms of osteoporosis and yet not be as obvious as in aged individuals.

Considerations Concerning Nutrition: It has long been suspected that prolonged low calcium intake might lead to osteoporosis in adult man. It was soon found, however, that many indi-

viduals have the ability to adjust to low calcium intake and that relatively few people develop severe or appreciable osteoporosis. There is, therefore, no proof that a dietary deficiency is the cause of this disorder in man, but some other endocrine dysfunction may arise from it in susceptible individuals. In experimental osteoporosis produced in birds on diets extremely low in calcium, the writer found that the adrenal glands were hyperplastic.[43] This observation suggests the possibility that a low calcium intake in an individual with a labile adrenal cortex may predispose him to develop osteoporosis. Of course, this leads to still other questions, but the point to emphasize is that the combination of calcium deprivation and adrenal cortical hyperactivity can exist together in nature under experimental conditions; this might be investigated further with some profit.

Protein Metabolism and Skin Disease: A common source of patients with osteoporosis and fractures in recent years is the Dermatology Clinic of every hospital where large numbers of patients with exfoliative dermatitis due to lymphoblastoma, acute allergies and psoriasis, or with sloughing skin due to pemphigus, are treated with prednisone and other cortisone derivatives. Under these conditions, there is an enormous protein rather than calcium draft with exogenous rather than endogenous adrenal hypercorticoidism and the result that gross osteoporosis can appear within 9 to 15 months. Such patients are comparable to molting birds with osteoporosis and fractures as described above. Molting, however, is believed to be under the control of the thyroid, but the subject deserves further study in relation to the adrenal.

Blood Chemistry: The serum levels of calcium, phosphorus and alkaline phosphatase are generally normal in experimental and clinical osteoporosis, but these tests are useful for excluding osteomalacia, osteitis fibrosa and Paget's disease. As the osteoporosis is largely a disorder of protein metabolism, it is reasonable to search for a variable and measurable change in some component of the plasma protein. Hypoproteinemia has been found in some cases, but this was the exception rather than the rule. Using the methods described by Schjeide and Urist[34] and Urist, Schjeide and McLean,[35] who observed the effects of estrogen on the serum proteins of birds, studies have been in progress on selected patients with and without postmenopausal osteoporosis. It was assumed that while gross chemical analyses of the serum protein showed no abnormalities, fractionation of the protein by physical methods such as paper electrophoresis and ultracentrifugation might show variations of some significance before and after estrogen therapy. The electrophoretic patterns were normal throughout. The ultracentrifugal analyses in six cases interpreted by the standard values for denser lipoproteins for normal individuals[36] showed values within the normal range in osteoporotic individuals. Patients under treatment with estrogen showed lipoprotein levels similar to those of normal pregnant women. Chylomicron and low density lipoprotein (so-called atherogenic group) were also generally the same in osteoporotics as in normal individuals of comparable ages.

Urinary Excretion of Hormones: The excretion of corticosteroids and gonadal hormones and their degradation products merits further study of 24-hour urine specimens of patients with osteoporosis. The inadequacy of present quantitative methods of measuring corticotrophin is most obvious. The adrenal cortical suppression test of Liddle, Coppage and Greene[37] seems worthy of a trial on patients with postmenopausal or senile osteoporosis. A study of the ACTH tolerance test in a significant number of patients with postmenopausal osteoporosis also seems necessary. The method of Norymberski,[38] which includes both 17-ketogenic steroids and 17-OH-corticosteroids, has not been informative in the writer's series of cases. The results of the chromatographic method of Brown[39] for quantitative fractionation of estrogen in the urine have been interesting. Like patients without osteoporosis studied by McBride,[40] patients with osteoporosis, after the menopause, showed normal levels of estrogen in the urine, a finding that would suggest that estrogen deficiency per se is not the cause of osteoporosis.

Treatment: It is now well established that large doses of estrogen and androgen will transform a negative nitrogen balance to a positive nitrogen balance, temporarily or for long periods of time, in many cases of osteoporosis of all kinds. The calcium and phosphorus balances generally show corresponding changes. This response is the same in Cushing's syndrome as in postmenopausal and senile osteoporosis.[9] The pain resulting from atrophy and deformity of the spine is relieved in some unknown way by these measures and by increasing the dietary intake of calcium, phosphorus

and vitamins and by corsets and braces to encourage normal ambulation. The patients seem to become relatively free of further fractures and in some instances show no further decrease in stature. However, the results of this regimen, while apparently better than those observed before Albright and Reifenstein's great contribution, remain to be improved. The writer has had 68 cases of osteoporosis of all forms under observation for 10 years and like other observers has not found a single case in which the density of the bone has been restored by treatment with sex hormones, estrogen or/and androgen. The lack of evidence of proliferative response in biopsy specimens of the bone tissue after treatment confirms the negative x-ray examinations.

The mode of action of estrogen in patients with osteoporosis is not as yet known. The possibility that it may suppress secretion of corticosteroid hormones from the adrenal cortex has strong support from our studies on birds and is now being investigated in patients. Riddle[41] corroborated Meckel's observation of 1896 that there is a great increase in weight of the adrenal glands in birds during the reproductive cycle. The same reaction occurs in mammals and can be reproduced by administration of large doses of estrogen. Although there seems to be no information about the effect on blood or urinary excretion of 17 hydroxycorticosteroids, changes in size, cellular structure, color and vascularity (brown degeneration) of the adrenal cortex itself[27] have been described following estrogen treatment in experimental animals. Inasmuch as the adrenal cortex in birds increased in size, chiefly across the zona reticularis, without any elevation in the blood sugar, the writer is moved to postulate that prolonged estrogen treatment of an adult individual suppresses the secretion of corticosteroids. If this is also true in man, sex hormone therapy in patients with osteoporosis may reasonably be regarded as a selective "medical decortication of the adrenal glands." Thus, the most that can be said for this form of treatment, as stated previously, is that it may arrest the progress of the disease and prevent further fractures and loss of height. The goal of clinical research must be to continue to try to find endocrine treatment that can restore the density of the bones. Adrenal gland biopsies for histochemical studies and subtotal adrenalectomy for treatment (to the extent of this writer's knowledge) have not been done in patients with either postmenopausal or senile osteoporosis. In such a way, it is necessary to prove or disprove by experiment the critical effect of the hormones of the adrenal cortex upon the development of osteoporosis and, hence, whether osteoporosis is pathognomonic of excess of endogenous adrenal cortical hormone, possibly hydrocortisone. The possibility that a potent ACTH inhibitor could be found that does not have inherent corticosteroid activity and also leads to a much more rapid restoration of bone mass has been considered by Reifenstein.[42] The most important need at the present is for an experimental animal with a disorder identical to clinical osteoporosis that can be used to test new agents. One of the most plentiful, long-lived and frequently castrated mammals is the female house dog, but as yet less is known about osteoporosis in "man's best friend" than about this most common disorder in man himself.

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NOTICES

Western Society for Clinical Research

The Western Society for Clinical Research will hold its twelfth annual meeting in Carmel-by-the-Sea, California, on Thursday afternoon, Friday morning and Saturday morning, January 29 through 31, 1959. Information may be obtained from Dr. William N. Valentine, Secretary-Treasurer, University of California Medical Center, Department of Medicine, Los Angeles 24, Calif.

Gastroenterology Research Group

The Gastroenterology Research Group will meet at 7:30 P.M. Friday, October 31, in the Walton Room of the Drake Hotel, Chicago. The general subject of the meeting will be "Absorption of Water and Electrolytes from the Gastrointestinal Tract." Preceding the meeting there will be a dinner in the French Room of the Drake at 5:30. All those interested are invited to attend both the dinner and the meeting. Information may be obtained from Dr. E. Clinton Texter, Northwestern University Medical School, Ward Memorial Building, 303 E. Chicago Ave., Chicago 11, Ill.

The Research Group will meet next spring on the afternoon of Thursday, June 4, at the Claridge Hotel in Atlantic City, N. J. The meeting will be a symposium on the topic "Fine Structures as Related to Transport, Absorption and Synthesis in the Gastrointestinal Tract."

Application for Membership

1. Young research workers are encouraged to apply for membership. It is unnecessary to await a member's invitation to join the AMERICAN FEDERATION FOR CLINICAL RESEARCH.

2. There is one requirement for regular membership: Publication of a meritorious investigation in clinical medicine or allied sciences. This should not be a case report or an abstract.

3. An applicant must ask a member of the Federation who knows him to sign his application.

4. Interested individuals should write to: AMERICAN FEDERATION FOR CLINICAL RESEARCH, 250 West 57th Street, New York 19, New York.

Conference on Electrical Technics in Medicine and Biology

The eleventh annual Conference on Electrical Technics in Medicine and Biology will be held in Minneapolis, Minnesota, November 19, 20 and 21. It is sponsored jointly by the Institute of Radio Engineers, The American Society of Electrical Engineers and the Instrument Society of America. The principal theme for the entire meeting will be "Biology and Computers." Special sessions will be devoted to the application of computers to electrocardiography and electroencephalography and to developing computer applications on the basis of information obtained from biology. Information may be obtained from Mr. Robert Erskine, Minneapolis-Honeywell, 2753 Fourth Ave. South, Minneapolis, Minn.

New Research Tools

The information reported here is obtained from manufacturers. All notices and inquiries should be addressed to **New Research Tools Editor, Samuel N. Turiel & Associates, Inc., 750 N. Michigan Ave., Chicago 11, Ill.** Include name(s) of the manufacturer(s).

- **New high voltage power supply** for use in spectrometry, with range from 500 to 2,000 volts. Unit is said to be compatible with all radiation detectors in use today. Tracerlab, Inc.
- **Gamma-sensitive scintillation detector** specifically designed for medical diagnostic applications of radioisotopes. It has three interchangeable collimators providing different directional characteristics. Nuclear-Chicago Corp.
- **Bulletin** discussing temperature control in gas and vapor chromatography. Burrell Corp.
- **Equipment for chromatography and electrophoresis,** including fraction collector, electrophoretic horizontal tank, electrophoretic constant current/constant voltage unit, electrophoretic densitometer and chromatographic tanks for strip, multi-sheet and two-dimensional paper chromatography. Baird and Tatlock (London), Ltd.
- **Equipment for automation in analysis,** including auto-titrator, Karl Fischer titrator, dispensing pipette unit, transfer pipette unit, recording absorptiometer and colorimetric analyser. Baird and Tatlock (London), Ltd.

An Urgent Request for Your Aid

The rapid growth of the Federation's services to members and to clinical research is inflicting "growing pains" of increasing severity. Between 1955 and 1956, *Clinical Research*, the official organ of the Federation, increased its published pages from 224 to 268. In 1957, this figure jumped to 324, and in 1958 we are currently publishing at the rate of over 500 pages per year.

The increasing costs of publishing our abstract journal, both in the number of pages and in cost per page, have thrown a serious financial burden on the Federation. It is apparent that these costs, together with other Federation operating expenses, considerably exceed our current income from membership dues. Because of the age bracket of our active membership, the Council has always resisted any proposal for a substantial increase in our dues.

Apart from membership dues, the Federation has two alternative sources of funds: (1) advertising in *Clinical Research* and (2) the dues of Sponsoring, Supporting and Contributing Memberships held by corporations, foundations and outside individuals. The list of these special members is shown on page 376 of the September, 1958 issue.

We should like to ask *your* cooperation, as a Federation member, in two specific ways:

1. If *you* have any friends or professional contacts among ethical pharmaceutical or medical supply firms or scientific instrument manufacturers not now represented in our advertising pages, please bring to their attention the advantages of their being among these advertisers and notify our National Office of any such contact you may be able to make.
2. If *you* are acquainted with a responsible officer of any of the types of firms described above, or of a foundation or a philanthropic individual who might be interested in aiding the unique services of the Federation in the field of clinical research by becoming a Sponsoring, Supporting or Contributing Member, please communicate this fact immediately to your undersigned Secretary-Treasurer who will consult with you as to the information required and the most appropriate way to bring the story of the Federation to his attention.

We must increase the operating funds of the Federation. Should we fail to do so during the current administrative year, it is inevitable that, to remain solvent, the Council will have to recommend an increase in the dues at the 1959 National Meeting. Because such a step would involve a constitutional amendment, any elevation in dues would require two years before any financial relief could be realized.

We earnestly request your help in meeting this urgent problem so vital to the welfare of your Federation.

George E. Schreiner, M.D.
Secretary-Treasurer

PROGRAM, MIDWESTERN SECTION

American Federation for Clinical Research

Thursday, October 30, 1958
Thorne Hall, Northwestern University, Chicago, Illinois

Dr. John F. Mueller, Presiding

Presentations will be limited to 10 minutes

9:00 A.M.

1. **Drug-Sensitive Chronic Hemolytic Anemia: Family Studies.**
   *William A. Newton, Jr. and Walter J. Frajola,** Columbus, Ohio. *page 392*
2. **The Effect of Lead Ingestion on the Daily Urinary Excretion of δ-Aminolevulinic Acid, Porphobilinogen, Coproporphyrin III and Lead.**
   *Donald E. Widmann,** Burritt W. Newton,* Irving Sunshine,* Robert C. Griggs and John W. Harris,* Cleveland. *page 393*
3. **Erythropoietic-stimulating Factor(s) and the Anemia of Chronic Renal Disease.**
   Neil I. Gallagher, John M. McCarthy* and Robert D. Lange,* Saint Louis. *page 391*
4. **Serum Turbidity Following a Fat Meal as a Test of Malabsorption.**
   J. D. Kabler, William H. Atwood, Jr.* and Robert F. Schilling,* Madison, Wisconsin. *page 410*
5. **The Inhibition of Streptococcal Diphosphopyridine Nucleotidase by Sera from Patients with Rheumatic Fever and Patients with Uncomplicated Streptococcal Pharyngitis.**
   Gerson C. Bernhard and Gene H. Stollerman,* Chicago. *page 412*
6. **The Effect of Tolbutamide on Glucose and Nitrogen Metabolism in the Totally Depancreatized Dog.**
   *Henry L. Wildberger and Henry T. Ricketts,** Chicago. *page 405*
7. **Metabolic Studies in Man of Chlorpropamide, a New Oral Hypoglycemic Agent.**
   *Kelly M. West, P. C. Johnson and Allen R. Hennes,** Oklahoma City. *page 406*

INTERMISSION

(10 minutes)

Refreshments, Courtesy of G. D. Searle & Co.

8. **Detection of the Heterozygous Carrier in Galactosemia.**
   David Yi-Yung Hsia, Irene Huang and Shirley G. Driscoll,** Chicago. *page 407*
9. **The Effect of Phenethylbiguanide on Pyruvate Utilization in Man.**
   *John A. Moorhouse, Stefan S. Fajans and Jerome W. Conn,** Ann Arbor, Michigan. *page 405*
10. **The Effect of Calcium Upon Small and Large Blood Vessels.**
    Francis J. Haddy, Chicago. *page 398*
11. **Intracellular Magnesium in Delirium Tremens and Uremia.**
    William O. Smith, Richard J. Warren and James F. Hammarsten,* Oklahoma City. *page 408*
12. **The Effect of pH on Norepinephrine-induced Contractions of Arterial Smooth Muscle.**
    Louis Tobian, Stephen Martin and William Eilers,** Minneapolis. *page 399*
13. **Tyrosine Deshalogenase Activity in Normal and Diseased Human Thyroid Tissues.**
    Edward A. Carr, Jr., William H. Beierwaltes,† Laurence L. Duncan, Norma R. Spafford and Roy A. Stambaugh,** Ann Arbor, Michigan. *page 404*
14. **Circulating Antibodies to Thyroglobulin: a Potential Mechanism in Multiple Thyroid Diseases.**
    Robert M. Blizzard, George J. Hamwi, Thomas G. Skillman and Warren E. Wheeler,** Columbus, Ohio. *page 404*

1:00 P.M.

Luncheon, Courtesy of the Upjohn Company

* By Invitation
† Senior Member

1:45 P.M.

BUSINESS MEETING, THORNE HALL

2:00 P.M.

15. **Studies on Gamma Globulin Deposition in the Human Kidney in Health and Disease.**
John H. Peters, Philip Freedman* and Robert M. Kark,** Chicago *page 414*
16. **Electromicroscopic and Ultramicrobiochemical Studies of the Potassium-depleted Kidney.**
*Robert C. Muehrcke and Sjoerd L. Bonting,** Chicago. *page 413*
17. **Asymptomatic Persistent Proteinuria: Studies by Renal Biopsy.**
Victor E. Pollak, Conrad L. Pirani, Robert C. Muehrcke and Robert M. Kark,** Chicago. *page 414*
18. **The Mechanism Regulating Pulmonary Capillary Blood Volume During Change in Position.**
Benjamin M. Lewis, William T. McElroy, Ernest J. Heyford-Welsing* and L. Carl Samberg,** Detroit. *page 397*
19. **The Pulmonary Abnormalities in Myxedema.**
William R. Wilson and George N. Bedell, Iowa City. *page 420*
20. **Studies on Airway Resistance in Chronic Pulmonary Disease.**
William E. Ruth and Charles E. Andrews, Kansas City, Kansas. *page 420*

INTERMISSION

(10 minutes)

21. **Measurement of Blood Flow in Minute Volumes of Specific Tissues in Humans.**
*James F. Schieve and Richard W. Stow,** Columbus, Ohio. *page 418*
22. **Mechanism of Norepinephrine-induced Pulmonary Congestion in Aortic Insufficiency.**
Timothy J. Regan, Valentino DeFazio, Kenan Binak and Harper K. Hellems, Detroit.* *page 398*
23. **Lipemia-induced Acceleration of Intravascular Clotting.**
Emanuel E. Mandel,† William Rosenthal and Harold Roth,** Chicago. *page 394*
24. **The Effect of Intravenous Fat Emulsions on Blood Coagulation.**
Joseph A. Werr and Frederick W. Preston,†* Chicago. *page 395*
25. **The Action of Heparin on the Conversion of Prothrombin and its Relationship to Accelerator Globulin.**
Emmanuel Mesel, Frederick Meyers* and Helen I. Glueck,†* Cincinnati. *page 396*
26. **Observations on the Law of Initial Values in Therapeutic Research: a Study of the Effects of Diuretic Agents.**
David C. Mock, Adrian Kyriakopoulos,* Mervin Clark, Edward N. Brandt* and James A. Hagens,* Oklahoma City.
page 417

Officers of the Midwestern Section

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Advance Reports Submitted to the Annual Meeting of the MIDWESTERN SECTION of the American Federation for Clinical Research

Thorne Hall, Chicago, Illinois · Thursday, October 30, 1958

BLOOD

Incidence and Significance of Tissue Mast Cells in Human Bone Marrow

By *Norman A. Nelson and Harold L. Oster.* Department of Medicine, Wayne County General Hospital, Eloise, Michigan.

Interest in tissue mast cells has been stimulated by recent findings implicating them in the metabolism of heparin, histamine, hyaluronic acid and serotonin. A review of the literature reveals not only controversy with respect to their function but disagreement as to their incidence and significance in various sites such as the marrow. Rohr encountered tissue mast cells in but a dozen marrow studies. Similarly, Fadem found them in only 7 out of 2,800 examinations. In contrast, Williams demonstrated tissue mast cells in 17%, Johnstone in 70% and Messerschmitt in 54% of the marrows studied. Their significance, even when present in large numbers, is likewise controversial.

In an attempt to evaluate further the incidence and significance of these cells, marrow smears obtained by aspiration were stained by the toluidine blue method of Undritz and examined for content of particles, fat spaces and mast cells. Tissue mast cells were found in 75% of 230 marrow examinations in 170 patients. Of the 184 specimens containing particles, tissue mast cells were identified in 94%. In 24, more than 6 of these cells were seen in a single oil immersion field.

An attempt was made to explain these findings and correlate them with clinical and hematologic data.

Increased Erythropoietin Activity in Plasma from Hypoxic, Nonpolycythemic Emphysematous Patients

By *Walter H. Whitcomb, Robert M. Bird, Philip C. Johnson, James F. Hammersten and Margaret Moore.* Radioisotope Service and Department of Medicine, V.A. Hospital and University of Oklahoma School of Medicine and Hospitals, Oklahoma City.

The study is concerned with the biologic assay for erythropoietin activity in the plasma from hypoxic and emphysematous patients who have failed to develop secondary polycythemia. The experimental plasmas were donated by 5 patients with obstructive emphysema. Each patient had hemoglobin values distinctly below that which would be expected in a normal individual chronically exposed to a similar degree of anoxia. Plasmas donated by two young men in vigorous, good

health served as controls. All plasmas were heated to 98°C. for 10 minutes, the supernatant fluid dialyzed and reconstituted to the original plasma volume. Mature male Sprague-Dawley rats were free fed and injected intraperitoneally daily for 13 consecutive days. The daily dose of plasma filtrate injected was equal to 2% of the animals current weight. The blood values were obtained immediately prior to the first injection and the day following final injection. Each group of rats receiving hypoxic plasma filtrates showed a mean increase in hemoglobin values. Rats receiving normal plasma or saline showed a variable response. A similar trend was noted in the erythrocyte counts and hematocrits. The increases in hemoglobin and RBC counts exhibited by the animals given hypoxic plasma were significantly different from the control value at the $p = < .02$ level. However, rats receiving hypoxic plasma filtrates showed a decrease in reticulocyte percentages. These results indicate in part that the plasma of patients who are hypoxic as a result of pulmonary emphysema but who have not developed secondary polycythemia contains a quantity of erythropoietin greater than that seen in normal subjects. This is interpreted as additional evidence that their erythropoietic unresponsiveness results from a refractory bone marrow.

Erythropoietic-stimulating Factor(s) and the Anemia of Chronic Renal Disease

By *Neil I. Gallagher, John M. McCarthy and Robert D. Lange.* St. Louis V.A. Hospital and Department of Medicine, St. Louis and Washington Universities.

In an attempt to clarify the mechanism of diminished erythropoiesis in patients with chronic renal disease, plasma levels of erythropoietic-stimulating factor(s) (ESF) were measured under various clinical and experimental conditions.

ESF assays were carried out in male Sprague-Dawley rats in which erythropoiesis had been depressed by starvation for 96 hours. Plasma for assay was acidified and boiled; the protein precipitate was washed and the supernatant collected, readjusted to pH 7 and concentrated by lyophilization. During the starvation period each animal was given two intravenous injections of plasma concentrate. One μc. of Fe^{59} was then injected and the 18-hour radioiron utilization determined.

Plasmas from 16 uremic patients with anemia were assayed. In six, assay was repeated after institution of oral cobalt therapy. Normal plasma and plasmas from nonuremic, chronically anemic patients were also assayed. Additional experiments were designed to determine if uremic plasma directly inhibited erythropoiesis or inactivated ESF. Normally fed animals were injected with concentrated uremic plasma and the 18-hour radioiron utilization was then determined. Plasma with high ESF activity was incubated with varying quantities of uremic plasma for 2 hours at 37°C. and then assayed.

Starvation for 96 hours reduces Fe^{59} utilization from $29.5 \pm 1.02\%$ (SDM) in the fed rat to $4.4 \pm 0.2\%$ (SDM). Normal plasma does not increase iron uptake in starved animals; plasmas from certain nonuremic, anemic patients increased Fe^{59} uptake in starved rats to between 12 and 18%. Plasma from 15 to 16 uremic patients with anemia did not increase Fe^{59} utilization. Cobalt improved the anemia without altering ESF content of uremic plasma. Uremic plasma did not inhibit Fe^{59} utilization in the fed rat or inactivate ESF.

These findings suggest that uremic plasma is deficient in ESF; this may explain in part the mechanism of erythroid failure in patients with chronic renal disease.

Myelotoxicity, Nephrotoxicity and Erythropoietic-stimulating Factor Production in Animals Treated with the Aminonucleoside of Puromycin

By *Robert D. Lange, Neil I. Gallagher, John M. McCarthy and Shirley Wright.* St. Louis V.A. Hospital and Department of Medicine, Washington and St. Louis Universities.

The possible role of the kidney in regulation of erythropoiesis remains controversial. The present studies were designed to investigate the bone marrow function and production of erythropoietic-stimulating factor(s) (ESF) in animals with a chemical nephrosis.

1.5 mg. of 6-dimethylaminopurine-3-amino-d-ribose per 100 Gm. body weight was given in 14 daily subcutaneous injections to immature male albino rats. Suitable controls were studied. A phenylhydrazine anemia was also produced in treated and control animals. In later studies the entire dose of aminonucleoside was given over a 24-hour period. Eighteen-hour radioiron utiliza-

tion experiments were performed by the method of Rambach et al. In vitro hemin synthesis using Fe^{59} and rabbit marrow was measured by a modification of the method of Thomas. ESF assay was carried out indirectly in starved rats by measuring radioiron utilization.

The production of nephrosis and uremia in rats by the aminonucleoside was confirmed. However, an erythroblastic depression of the marrow occurred in treated animals together with a reticulocytopenia. Aminonucleoside-treated animals given phenylhydrazine developed a reticulocytosis of 7.9% compared to 48% in controls given phenylhydrazine. Iron utilization by RBC was decreased 1.2% in aminonucleoside animals as compared to 26.5% in controls. Similar results were found in animals given only two injections of aminonucleoside and before the onset of uremia. The aminonucleoside suppressed in vitro hemin synthesis. In assays for ESF, control phenylhydrazine plasma increased Fe^{59} utilization of starved rats from 4.4% to 13.3%. Plasma from animals pretreated with aminonucleoside and then given phenylhydrazine increased Fe^{59} utilization to 7.3%.

These results indicate that the aminonucleoside has a myelotoxic action in addition to its nephrotoxicity and that animals made nephrotic by its use are less able to produce ESF and respond to the stimulus of anemia.

Drug-Sensitive Chronic Hemolytic Anemia: Family Studies

By *William A. Newton, Jr. and Walter J. Frajola.* Departments of Pathology and Medicine, Ohio State University and Children's Hospital, Columbus, Ohio.

We have studied 3 white boys who have a chronic nonspherocytic anemia manifested first in the newborn period by unexplained hyperbilirubinemia and later by a continuing mild to moderate anemia with increased reticulocytosis. All have had episodes of hemoglobinuria, occurring either spontaneously or following specific drugs, particularly sulfa preparations. Their red cells have been shown to be markedly deficient in glucose-6 phosphate dehydrogenase and show pathologic levels of reduced glutathione after incubation with acetyphenylhydrazine.

Heretofore, this defect has been described as occurring in apparently normal individuals who developed an acute self-limited hemolytic process following ingestion of certain drugs or fava beans. Family studies of these patients have shown that this defect occurs chiefly in Negroes and is inherited in a sex-linked recessive pattern. Studies of the families of these 3 boys have failed to demonstrate any definite genetic source of the glucose-6 phosphate dehydrogenase deficiency in siblings, parents and, in some instances, more distant relatives.

Thus, even though the chemical studies on their erythrocytes are comparable to patients who show no anemia or increased hemolysis except when exposed to the specific drugs or fava beans, these children are different in 2 ways. First, they present the hematologic picture of a chronic nonspherocytic hemolytic anemia with or without specific drug exposure, and family studies do not show a genetic source of their enzyme defect. The reduced glutathione levels were performed by the method of Gruenert and Phillips; the glutathione stability test of Beutler et al. was used; and the glucose-6 phosphate dehydrogenase activity was determined by the method described by Zinkham et al.

Transient Hemolytic Anemia Associated with Liver Disease

By *Francis J. Holt and Donald R. Korst.* Radioisotope Service, V.A. Hospital and Department of Medicine, University of Michigan Medical School, Ann Arbor, Michigan.

A transient hemolytic anemia associated with hyperlipemia and/or hypercholesterolemia and mildly disturbed hepatic function in which the anatomic diagnosis on liver biopsy was usually minimal to moderate portal cirrhosis has recently been described by Zieve. This type of anemia has been studied in 5 patients during the past 2 years by means of red cell survival times using Cr^{51}-tagged cells.

The patients were admitted to the medical service where liver function tests and hematologic studies, including repeat hemoglobin, hematocrit and reticulocyte counts, were done. A Cr^{51} RBC survival time and external counting for splenic activity was done in each patient and repeated in 2 patients at a later date. Liver biopsy was done in 4 patients and showed early to minimal changes of portal cirrhosis in 3. Bone marrow aspiration was performed in 3 patients and

showed erythroid hyperplasia of moderate to marked degree in each case. A history of chronic, excessive alcoholic intake was obtained in 4 patients.

Serum cholesterol values ranged from 1050 mg.% to 219 mg.% with a mean of 490 mg.%. Lowest hemoglobin and hematocrit values in each patient varied from 9.7 Gm. and 29% to 11.4 Gm. and 35%, respectively. Highest reticulocyte counts in each patient ranged from 2% to 15%. Hemoglobins and hematocrits and reticulocyte counts returned to normal or improved levels in from 1 to 8 weeks in each patient. RBC T/2 survival times by the Cr^{51} method varied from 17 days to 10 days (mean 13.8 days), compared to a normal of 28 to 32 days in this laboratory. External counting done in each patient did not reveal excessive splenic localization to indicate a splenic mechanism for the anemia. The follow-up studies in 2 patients indicated presence of shortened cell survival, but of a lesser degree than when first studied.

A form of hemolytic anemia associated with liver disease and usually with an elevated cholesterol has been confirmed in 5 cases by Cr^{51} studies. This appears to be of a transient nature and is improved by hospital care.

The Effect of Lead Ingestion on the Daily Urinary Excretion of δ-Aminolevulinic Acid, Porphobilinogen, Coproporphyrin III and Lead

By *Donald E. Widmann, Burritt W. Newton, Irving Sunshine, Robert C. Griggs and John W. Harris.* Departments of Medicine and Pharmacology, School of Medicine, Western Reserve University, Cuyahoga County Coroner's Office and Cuyahoga County Hospital.

To determine the time relationships among the changes in the excretion of δ-aminolevulinic acid (ALA), porphobilinogen (PBG), coproporphyrin III and lead, total daily urine collections were made from 2 normal volunteers before, during and after a period of measured lead ingestion. Corresponding determinations were made on 8 patients while receiving Versene therapy for acute lead intoxication. Methods employed were: ALA and PBG, Mauzerall and Granick; coproporphyrin III, Schwartz and coworkers; lead, Bessman and Layne.

Volunteer I: During a 17-day ingestion period and 18-day recovery period, ALA excretion progressively increased exceeding upper limits of normal from day 9 through 35; lead excretion was abnormal from day 10 through 35; coproporphyrin from day 12 through 27; PBG only on day 15.

Volunteer II: During a 32-day ingestion period and a 19-day recovery period, ALA excretion progressively increased exceeding normal limits from day 9 through 51; lead excretion was abnormal from day 21 through 40; coproporphyrin III from day 28 through 37; PBG excretion never became abnormal.

In neither volunteer were hematologic alterations detected (CBC, reticulocytes, stippled count, osmotic and mechanical fragility).

In the 8 patients, although lead excretion was augmented during Versene therapy, ALA and coproporphyrin III levels rapidly decreased, presumably indicating a detoxification effect.

In both volunteers the excretion of ALA exceeded normal before that of the other heme precursors or lead. After discontinuing lead ingestion, coproporphyrin levels quickly returned to normal while ALA excretion remained elevated even when the urinary lead excretion had returned to normal. During Versene therapy, ALA and coproporphyrin output decreased rapidly despite increased lead excretion. Thus, it would appear that ALA determinations provide the more sensitive indicator of the biochemical disturbance of heme synthesis in both the initial and continuing manifestations of lead intoxication.

Leukocytic Function in Various Hematologic Disorders: A Preliminary Report

By *Norman A. Nelson, A. F. White and Virginia Bodick.* Department of Medicine, Wayne County General Hospital, Eloise, Michigan.

The recent studies of Osgood, Craddock, Yoffey and others, emphasize that the location of the vast majority of leukocytes in the body paradoxically is outside the blood and blood-forming tissues. Osgood's calculations suggest that the ratio of neutrophils outside the blood and hematopoietic tissues to those circulating in the blood is of the order of 60 to 1. The constancy, then, of the leukocyte count found in the normal peripheral blood becomes astounding. To achieve this, a highly efficient homeostatic mechanism must be operative. A fine balance must be maintained between production, release from sites of

production, circulating forms, cells outside blood and blood-forming tissues, their interchange and their utilization or destruction in these various locations.

Evaluation of abnormalities in leukocytes would appear incomplete if due consideration were not paid to the vast numbers present outside the blood. Although various elaborate technics have been suggested and used in attempting to assess this aspect, the simple technic introduced by Rebuck over 10 years ago is most intriguing and readily applicable clinically. It was hoped that this might provide not only further information concerning morphologic and functional aspects, as has been so strikingly documented by Rebuck, but that in addition it might prove to be a means of studying extravascular leukocytes.

The technic used, being that first introduced by Rebuck with minor modifications, is described. Patients with various hematologic disorders, including idiopathic extramedullary hematopoiesis, leukopenia with monocytosis of undetermined etiology, chronic lymphatic leukemia undergoing transition to myeloid metaplasia and leukopenia associated with hepatic cirrhosis, were studied.

The results to date suggest: (1) that means of evaluating extravascular leukocytes are important; (2) that this may be readily accomplished by the technic of Rebuck; and (3) that the findings can be most interesting and rewarding.

Platelet Tansfusion Utilizing ACD-Blood in Plastic Bags from Routine Storage

By *John R. Tobin and Irving A. Friedman.* Division of Medical Education and Department of Hematology, Hektoen Institute for Medical Research, Cook County Hospital, Chicago.

This study demonstrates the utilization of "bank blood" for rapid procurement of platelet transfusions.

The effects of these platelet transfusions were studied in 10 patients displaying thrombocytopenia with bleeding due to diverse etiologies, including lymphosarcoma involving marrow, acute leukemias, multiple myeloma, metastatic carcinoma to marrow and hypoplasia of marrow.

Four plastic bag units of whole blood from normal donors, compatible with the recipient and in storage less than 48 hours, were selected. The four units were centrifuged at 1200 rpm for 15 min. at room temperature. The supernatant, platelet-rich plasmas were transferred by a positive-pressure press into smaller plastic bags (transfer packs). The connecting tubes were clamped and the connected packs were centrifuged in the same cup at 2500 rpm for 20 min. After centrifugation, there is a thick, whitish residue (platelets) in the bottom of each transfer pack. The supernatant platelet-free plasmas are pressed back into the original blood packs. Approximately 30 ml. are allowed to remain in each transfer pack for resuspension of the platelet residue. The four transfer packs are connected in tandem by "plasma transfer sets" and all of the platelets are pressed into a single pack. If whole blood is desired, all of the material is pressed into a single blood pack.

Clinical response, measured by cessation of bleeding, was definite in 83% (10). An increment of the platelet count in excess of 60,000 was obtained in 50% (6). The bleeding time was reduced to normal in 50% (6) and in 4 instances remained normal for over 92 hours. Clot retraction was abnormal before transfusion in all instances and became "complete" in 66.6% (8). Prothrombin consumption revealed an increment of over 10 sec. in 41% (5). Best results were evident in patients with hypoplastic marrows.

This study demonstrates that platelet transfusions prepared from plastic bag "bank blood" are efficacious.

Lipemia-induced Acceleration of Intravascular Clotting

By *Emanuel E. Mandel, William Rosenthal and Harold Roth.* Department of Medicine, Chicago Medical School and Mount Sinai Hospital, Chicago.

The effect of intravenous administration of a fat emulsion (Don Baxter, Inc.) upon clotting of stagnant venous blood was studied. The jugular and femoral veins of anesthetized dogs were surgically freed and each vein divided into several segments by applying seraffine clamps (Wessler's technic). Vein segments were removed at 5-minute intervals and their contents carefully checked for presence of a fibrin clot. The time that elapsed from the moment of clamping to the first appearance of a clot was noted. In 21 experiments on 15 fasting dogs, this clotting time was 30 minutes or longer. In 20 experiments on 13 dogs, 20 to 40 ml. of fat emulsion were infused into an antecubital vein prior to clamping;

clotting times ranged between 10 to 25 minutes. In 8 of these 13 dogs, two "clamping" experiments were performed after the fat infusion—the first one, on the right side, 1 to 10 minutes later, the second one, on the left, 30 to 45 minutes later. In each of these infusion experiments, clotting times on the left (second) side were 5 to 10 minutes longer than on the right (first) side, coinciding with a decrease in serum turbidity and fatty acid content from the markedly elevated levels precipitated by the infusion. In the 5 remaining dogs, lipemia was induced *after* the first "clamping" experiment and accompanied by a substantial decrease in clotting times over the respective preinfusion values. These in vivo results tend to confirm the phenomenon of lipogenic hypercoagulability previously demonstrated by in vitro methods.

The Effect of Intravenous Fat Emulsions on Blood Coagulation

By *Joseph A. Werr and Frederick W. Preston.* Department of Surgery, V. A. Research Hospital, Chicago.

Daily intravenous infusions of fat emulsions for 20 days or more have been reported to cause hemorrhage, blood coagulation defects, thrombocytopenia and anemia in some patients. Because of these observations, clotting and other tests were done before, during and after a series of 21 daily intravenous infusions of 500 ml. of fat emulsion (Lipomul-I.V.) into 20 patients hospitalized for a variety of benign and malignant diseases.

The following tests were included: (1) whole blood clotting time; (2) plasma recalcification time; (3) fasting serum turbidity; (4) prothrombin time; (5) platelet count; (6) fibrinolysin activity; (7) clot retraction and quality of clot strength; (8) Hgb.; (9) hematocrit; (10) W.B.C.; and (11) urinalysis.

All patients developed one or more coagulation defects. Eighteen patients developed prolonged whole blood coagulation times. In one patient the coagulation time was 5 hours on 1 occasion. The plasma recalcification time tended to become prolonged, although after the first few infusions a slight decrease in recalcification time was often observed. The fasting serum turbidity was increased in most patients after many infusions. The prothrombin time was depressed in 13 patients, the lowest value being 11%. Platelets were decreased in approximately half the patients. Fibrinolysin activity tended to become increased with multiple infusions. Clot retraction tended to decrease, and clot strength decreased. Routine urinalysis revealed the presence of RBC in several specimens. This later cleared after the series of infusions was completed. Three patients developed severe hemorrhage sufficient to cause shock requiring blood transfusions. Most patients became anemic, which in some was attributed to carcinoma.

Daily intravenous infusions of fat emulsion can be given safely for 2 weeks, but coagulation defects may develop if given for longer periods.

The Effect of a New Water-Soluble Derivative of Vitamin K_1 (MK-112) on Drug-induced Hypoprothrombinemia

By *Giles Bole and Ivan F. Duff.* University of Michigan, Ann Arbor, Michigan.

Three healthy adult males were given Dicumarol (1100, 800, 650 mg.) on three separate occasions. Spontaneous recovery from drug-induced hypoprothrombinemia was then compared with the effect of intravenous administration of vitamin K_1 (2-methyl-3-phytyl-1, 4-napththoquinone), Mephyton and a new water soluble derivative, dihydrovitamin K_1 (2-methyl-3-phytyl-1, 4-naphthohydroquinone-1, 4-diphosphate).

"Prothrombin" was measured by the one-stage (Quick) and two-stage (Seegers) methods. Factor V (Wolfe) and Factor VII (Owren) determinations were also made. "Prothrombin" time (Quick) prolongation to at least twice control values was achieved, after which the preparations of vitamin K_1 were injected intravenously in a dose ranging from 0.5 mg. to 1.0 mg. per Kg. of body weight.

In healthy subjects significant effect on "prothrombin" time was noted within one hour; Factor VII levels were satisfactorily altered in 1½ hours after injection of dihydrovitamin K_1. Dihydrovitamin K_1 was also found to be very active in counteracting the excessive anticoagulant effect of oral Coumadin in a 78-year-old woman with profuse gastrointestinal hemorrhage. Two hours after intravenous injection of 20 mg. the "prothrombin" time had been reduced from the initial value of 93.6 sec. to 49.0 sec.; 5 hours after a total of 60 mg. the "prothrombin" time was 22 sec. In a 48-year-old woman with prolongation to 57.2 sec. (Quick) as a result of Dicumarol

therapy, 40 mg. of dihydrovitamin K_1 intravenously resulted in reduction to a safe level of 16.1 sec. within 3 hours.

It is concluded from these preliminary studies that water soluble dihydrovitamin K_1 is as effective as oil-soluble vitamin K_1 in reversing Coumarin-induced hypoprothrobinemia. No local or constitutional reactions to the intravenous use of this new preparation were noted.

The Action of Heparin on the Conversion of Prothrombin and its Relationship to Accelerator Globulin

By *Emmanuel Mesel, Frederick Meyers and Helen I. Glueck.* Departments of Medicine and Obstetrics, University of Cincinnati College of Medicine and Cincinnati General Hospital.

These studies concern the effects of heparin on prothrombin conversion. Normal oxalate or citrate plasmas were incubated at 37°. The one-stage prothrombin time (O.S.P.T., thromboplastin-calcium clotting time) was repeated at intervals. Incubation of heparinized plasma prolonged the O.S.P.T., oxalate plasmas showing greater change than citrated samples. Within limits, the effect depended on the amounts of heparin in the incubation mixture.

Plasmas deficient in accelerator globulin (Ac-g, V, labile factor) were more sensitive to incubation with heparin than the same samples before destruction of the accelerator by heat. Incubation prolonged the O.S.P.T. in a linear fashion, until a plateau was reached, further incubation no longer affecting the O.S.P.T. Addition of an excess of beef serum Ac-g to heparinized plasma deficient in the accelerator restored the O.S.P.T. to normal levels. This sample on incubation showed no prolongation of the O.S.P.T. With lesser quantities of accelerator, incubation lengthened the O.S.P.T. These changes were independent of the heparin effect on the thrombin time (T.T.), i.e., the conversion of fibrinogen to fibrin by thrombin. The T.T. of heparinized Ac-g deficient plasma was prolonged. The latter reaction was immediate, remaining unaltered with incubation. The two-stage and TAMe synthetic substrate assays of prothrombin revealed smaller yields of thrombin from prothrombin when normal plasma was incubated with heparin. The findings of the TAMe assay resembled those noted previously with Ac-g deficient plasma.

Dog plasma containing excessive amounts of Ac-g required 10 times as much heparin as human plasma to produce the effects described.

Intravenous injection of heparin in normal subjects produced the following results: 10-minute sample, prolonged O.S.P.T. increasing with incubation, and prolonged clotting time; 2-hour sample, prolonged clotting and T.T., essentially normal O.S.P.T. not increasing on incubation.

Our studies confirm and extend those of Brambel et al. regarding these functions of heparin.

BLOOD ENZYMES

Lipoprotein Lipase in Hepatic Disease and Myocardial Infarction and Its Relationship to Serum Lipids and Hypoalbuminemia

By *Saul P. Baker, Harold Levine, Liebert Turner and Alvin Dubin.* Department of Medicine, Chicago Medical School and Hektoen Institute for Medical Research, Cook County Hospital, Chicago.

A *decreased* heparin-activated clearing-factor (lipoprotein lipase) response has been associated with coronary atherosclerosis. Utilizing a standardized in vitro method, a significantly *increased* clearing-factor response has been observed in patients with primary diagnoses of chronic alcoholism and/or Laennec's cirrhosis. Inasmuch as a lesser incidence of atherosclerosis has been postulated in Laennec's cirrhosis, more extensive studies have been made on a larger group, comprising 108 patients to date, with various types of hepatic pathology, jaundice or recent myocardial infarction. Twenty-nine of these subjects demonstrated clinical and laboratory evidence of Laennec's cirrhosis; 11 had hepatitis (IH or SH); 6 had obstructive jaundice; and 12 had suffered a recent myocardial infarction. Comprehensive laboratory evaluation of hepatic status included BSP and serum bilirubin.

These studies have confirmed the presence of an *increased* lipoprotein lipase response in patient's with Laennec's cirrhosis. Total serum

lipids in these subjects generally ranged *below* 600 mg./100 ml., lipid phosphorus *below* 10 mg./100 ml. and total cholesterol *below* 175 mg./100 ml. Per cent of serum cholesterol esters was almost uniformly low, being *below* 40% (below 75 mg./100 ml.) In contrast, the patients with hepatitis, obstructive jaundice or recent myocardial infarction demonstrated a *"normal"* or *decreased* lipoprotein lipase response. Generally, total serum lipids in these subjects ranged *above* 600 mg./100 ml. and total cholesterol *above* 175 mg./100 ml. (myocardial infarction and obstructive jaundice). The serum cholesterol/lipid phosphorus ratio appeared to be related to the clearing-factor response in Laennec's cirrhosis and myocardial infarction. Hypoalbuminemia produced no depressant effect upon lipoprotein lipase, the *greatest* response being observed with serum albumin *below* 3.0 Gm./100 ml.

These results suggest that parenchymal liver cell damage, either acute or chronic, may be reflected by the concentration of heparin-activated lipoprotein lipase in plasma. A possible interrelationship may also exist between clearing factor response, cholesterol/lipid phosphorus ratio, lipid metabolism and atherosclerosis.

CARDIOVASCULAR SYSTEM

Direct Spicardial Potentials in Right Ventricular Hypertrophy

By *Richard H. Wasserburger, William P. Young, Karl Siebecker, Jr. and D. Joseph Freeman.* V. A. Hospital and University Hospitals, Madison, Wisconsin.

Based upon previous electrocardiographic analysis of 400 cases of established congenital heart disease by means of cardiac catheterization, it was observed that the most common electrocardiographic expression of right ventricular preponderance consisted of an rR′ pattern in the right precordial leads which was subtended to 3 variable points, AV_R, $V4_R$ and V_E.

In an attempt to further define the origin and significance of the various right precordial QRS complex patterns in established instances of right ventricular preponderance, direct right and left ventricular epicardial potentials have been recorded from predetermined areas on 12 patients with isolated valvular pulmonic stenosis and tetralogy of Fallot. With but a single exception, a distinct rR′ pattern has been identified as arising from the right base of the high outflow tract of the right ventricle. Most interestingly, as one approaches the apex of the right ventricle with the exploring electrode, an rS pattern is identified similar to that seen in augmented limb lead AV_L. Also, the timing of the peak of the R′ wave of the right ventricular leads occurs later than the peak of the R wave recorded over the left ventricular surface. This refutes the premise that the tall R′ waves seen in the conventional right precordial leads stem from a posteriorly displaced left ventricle.

The Mechanism Regulating Pulmonary Capillary Blood Volume During Change in Position

By *Benjamin M. Lewis, William T. McElroy, Ernest J. Heyford-Welsing and L. Carl Samberg.* Departments of Medicine, Detroit Receiving Hospital and Wayne State University College of Medicine.

The volume of blood in the pulmonary capillaries (V_C) can be calculated by measuring the diffusing capacity of the lungs for carbon monoxide ($D_{L_{CO}}$) at 2 or more alveolar oxygen tensions. We have made such measurements using a rebreathing technic in 6 normal subjects while recumbent and during head-up tilt at 45°. $D_{L_{CO}}$ invariably fell on tilting. In 5 of 6 subjects the decrease in $D_{L_{CO}}$ was due chiefly to a decrease in V_C averaging 18.8%. To investigate the mechanism of this decrease, tilting was repeated in these subjects during the infusion of a rapidly acting ganglionic blocking agent (Arfonad). All 6 subjects, including the one whose V_C had not changed on tilting during the control study, now demonstrated a lower V_C on tilting. The average decrease from the recumbent position was 23.7%, and the V_C when tilted during Arfonad was 24.2% less than the V_C when tilted during the control period. In 5 of these subjects tilting was repeated a third time while norepinephrine was infused. No significant decrease of V_C occurred on tilting. When recumbent there was no significant increase in V_C during norepinephrine as compared with the control. These findings suggest that V_C is a function of venous return, or more directly of right ventricular output, since the normal response to tilting was

exaggerated by ganglionic blockade that would encourage venous pooling and is blocked by infusion of norepinephrine which produces intense vasoconstriction that would decrease venous pooling.

Mechanism of Norepinephrine-induced Pulmonary Congestion in Aortic Insufficiency

By *Timothy J. Regan, Valentino DeFazio, Kenan Binak and Harper K. Hellems.* Cardiovascular Laboratory, Department of Medicine, Detroit Receiving Hospital and Wayne State University College of Medicine, Detroit.

The influence of the sympathetic nervous system upon cardiovascular dynamics in normals and in patients with aortic regurgitation has been simulated by the infusion of norepinephrine (0.2 gamma/Kg./ min. for 15 minutes). Simultaneous with similar increases in arterial pressure in both groups, the most striking response in the 7 patients with isolated aortic valve insufficiency and compensated hearts was an elevation of the pulmonary "capillary" pressure from 9 to 30 mm. Hg ($p = <0.001$) in contrast to a 5 mm. rise in 5 normals. Whereas there was a slight decrease in heart rate and cardiac output in normals (6.4 to 5.9 L./min.), the aortic insufficiency group had no significant change in rate and considerable depression of total cardiac output (8.74 to 6.74 L./min.[$p = <0.01$]). While the forward flow (Hamilton Method) fell from 5.14 L./min. to 4.70 L./min. ($p = <0.05$), the regurgitant flow (Korner-Shillingford, down-slope of indicator dilution curve) fell from 3.51 to 1.54 L./min. ($p = <0.01$). This represented a change from 66% to 40% of forward flow.

To test the postulate that the elevated hydrostatic pressure in the pulmonary "capillary" represented pulmonary congestion due to blood displacement from the periphery, 3 patients received norepinephrine after tourniquets were applied on 3 extremities. Despite the usual arterial pressure increase, the pulmonary "capillary" pressure did not rise above normal. Mean regurgitant flow increased from 3.17 L./min. to 4.01 L./min. while forward flow diminished slightly from 5.25 L./min. to 5.13 L./min.

Thus, the hemodynamic response to norepinephrine in aortic insufficiency appears to depend upon displacement of blood to the pulmonary vascular bed and to the grossly enlarged heart unable to respond to elevation of filling pressures with an increase in cardiac output. The paradoxical decrease in regurgitant flow in the face of elevated arterial pressures may be largely explained on the reduced aortic-ventricular diastolic pressure gradient and diminished forward flow.

The Effect of Calcium Upon Small and Large Blood Vessels

By *Francis J. Haddy.* Departments of Medicine and Physiology, Northwestern University and V. A. Research Hospital, Chicago.

Hypertension is frequently associated with hyperparathyroidism, vitamin D intoxication and excessive administration of calcium salts. In order to determine whether the hypertension might be related to a direct action of the calcium ion upon blood vessels, 10% solutions of calcium chloride, calcium lactate and calcium gluconate were infused into the brachial arteries of 31 dogs. The rate of blood flow to the forelimb was held constant at an average value of 80 ml./min. Pressures were measured in the brachial artery, a small artery in the foot pad, a small vein in the paw and in the cephalic vein. Brachial and small arterial pressures increased as a function of infusion rate with all three calcium salts. Venous pressures were unaffected. In 10 animals, average serum calcium concentrations in the cephalic vein of 10.9, 13.1 ($CaCl_2$ 0.1 ml./min.) and 23.0 mg.% ($CaCl_2$ 0.33 ml./min.) were associated with potassium concentrations of 3.4, 3.2 and 3.2 mEq./L., brachial arterial pressures of 142, 149 and 168 mm. Hg, small arterial pressures of 101, 106 and 121 mm. Hg, small vessel (mainly arteriolar) resistances of 1.19, 1.24 and 1.43 mm. Hg/ml./min.(PRU), arterial resistances of 0.56, 0.60 and 0.65 PRU and total resistances of 1.82, 1.90 and 2.14 PRU. Sodium concentration in the foreleg and calcium concentration in the body as a whole did not change. The response of brachial arterial pressure to a challenging dose of ¼ μg. norepinephrine into the brachial artery was lessened by $CaCl_2$ in 15 of 19 experiments. The response to ½ μg. acetyl-beta-methylcholine chloride did not change. Ten % solutions of sodium and potassium salts dilate. Therefore, the local action of the calcium ion is to constrict arteriols and large arteries through some mechanism other than increasing the sensitivity of the vessels to norepinephrine.

The Effect of pH on Norepinephrine-induced Contractions of Arterial Smooth Muscle

By *Louis Tobian, Stephen Martin and William Eilers.* Department of Medicine, University of Minnesota School of Medicine and University of Minnesota Hospitals, Minneapolis.

Spirally cut strips of rat aorta were mounted under .5 Gm. tension in Krebs-Henseleit medium with 25 mEq. bicarbonate/L. The bath was alternately aerated with gas containing either 4.5 or 11% CO_2, giving pH's of 7.5 and 7.2, respectively. These alternating changes of pH did not by themselves change the tension of the strips. Then norepinephrine (10^{-9}) was added to the bath at pH 7.5 and the increased tension was measured isometrically. The norepinephrine was then washed out, allowing the tension to return to the .5 Gm. baseline; the pH was changed to 7.2 and norepinephrine (10^{-9}) was again added. These alternations of pH were continued consecutively for 5 successive contractions in each of 6 strips. At pH 7.5, the strips developed an average additional tension of .30 Gm. with norepinephrine; while at pH 7.2, the average additional tension was only .17 Gm. The acid medium caused an inhibition of contraction in every strip, averaging 43% inhibition. In a similarly arranged study with 6 other strips, the pH was alternately changed from 7.5 to 7.2 by alternating media with either 28 mEq. or 14 mEq. of bicarbonate per liter, respectively, while keeping the CO_2 constant. Again the average increase in tension with norepinephrine at pH 7.5 was 37% greater than that at pH 7.2. In 3 other strips the pH was alternated between 7.35 and 7.15 by changing the bicarbonate concentration while keeping the CO_2 constant. The increase in tension with norepinephrine was 38% greater at 7.35 than at 7.15. In summary, in every strip of every study the concentration was invariably diminished in the more acid medium ($p = .00001$). This would suggest that acidotic patients can be rendered more sensitive to norepinephrine by correcting their acidosis.

Reflex Venoconstrictor Response to Strong Autonomic Stimulation

By *Joseph C. Ross, William P. Wilson and John B. Hickam.* Department of Medicine, Indiana University School of Medicine, Indianapolis.

There is much evidence that venous system vasomotor activity is important in the over-all circulatory regulation. Normally, a lowered blood pressure, as produced by the Valsalva maneuver, is attended by reflex peripheral vasoconstriction, resulting in increased venous return to the heart. This response has utility in counteracting circulatory disturbances such as blood loss, but may be disadvantageous in congestive failure. The purpose of this investigation was to determine how great a venoconstrictor response could be obtained, as reflected by central venous pressure (CVP), when the autonomic nervous system was strongly stimulated by electric shock therapy (EST).

CVP was measured by strain gauge from a catheter in the superior vena cava during and for a period following EST in 13 physically normal psychiatric patients. Motor response was virtually eliminated by 10 to 60 mg. succinyl choline. Medication also included atropine (0.8-1.0 mg.), oxygen and enough Pentothal to abolish the wink reflex. Eleven patients received brief stimulation (1 to 7 seconds) and 2 received EST for 60 to 90 seconds.

Ten of 11 patients had an increased CVP, with the increase beginning within 5 seconds after EST in 9 patients. Mean increase after brief stimulation was 5.3 (S.D.2.3.) mm.Hg and, in 9 subjects, the peak occurred within 20 seconds after EST was started. In 2 patients (4 determinations) where longer EST was given, a rise in CVP began promptly and the peak was reached within 20–120 seconds. The mean increase in CVP in these 4 cases was 13.0 (S.D.3.1) mm.Hg. In some cases, increases were maintained for as long as 5 minutes after the EST.

The prompt rise in CVP indicates that venoconstriction was at least partially reflex, although the prolonged elevation after EST may be humoral in origin. The large magnitude of the pressure increase is comparable to that seen in congestive heart failure.

Venomotor Changes in the Forearm of Man after Atropine Administration

By *A. W. Horsley and John W. Eckstein.* Cardiovascular Research Laboratories, Department of Internal Medicine, State University of Iowa College of Medicine, Iowa City.

Recent observations suggest that atropine induces venous pooling. This would inply a reduction in venous tone since venous pressure de-

creases. The experiments reported here were done to measure the tonic state of the forearm veins in man after atropine administration.

Normal young men were studied in the supine position. Venous pressure–volume curves were obtained plethysmographically from the right forearm. Pressure was measured in the antecubital vein of the dependent left arm. Observations were made before and after intravenous administration of atropine sulfate (2.0 mg.).

Active venous constriction occurred in each of 9 experiments; the forearm venous volume at a transmural venous pressure of 30 mm. Hg averaged 4.2 ml./100 ml. of tissue during control periods and 3.5 ml. after atropine. Venous pressure fell appreciably in 7, slightly in 1 and remained unchanged in 1 of the 9 tests; the average pressure change was a fall of 1.7 mm. Hg. Blood shifted from the forearm veins in each experiment. The natural venous volume of the forearm averaged 2.9 ml./100 ml. of tissue during control periods and 2.1 ml. following atropine. This shift was caused by the active increase in venous tone as well as the fall in venous pressure. The maximum change in venous tone occurred about 24 minutes after administration of the drug. The maximum fall in venous pressure occurred slightly later.

The observations of Berry and co-workers form strong evidence to support their suggestion that atropine induces venous pooling. It is probable that venous dilatation occurs in the splanchnic or in some other vascular bed. Dilatation does not, however, occur in the veins of the forearm.

Blood Lipid Levels Following the Administration of Supplementary Unsaturated Fatty Acids

By *A. A. Kyriakopoulos, D. C. Mock and J. F. Hammarsten.* Medical Service, V.A. Hospital and Experimental Therapeutic Unit and Department of Medicine, University of Oklahoma School of Medicine, Oklahoma City.

Unsaturated fatty acid preparations and lipotropic agents are currently advertised as effective in lowering blood lipids when given as a supplement to a normal diet. Evaluation of the effectiveness of such therapy was investigated in 9 male patients with angina who had elevated blood cholesterol values. All subjects were followed as outpatients. Blood samples (fasting) were drawn at the same hour at weekly intervals. Total cholesterol, phospholipids, serum lipoproteins (ultracentrifuge) and serum proteins (electrophoresis) were determined. Diet content was calculated daily from a diary kept by each patient. There was very little intraindividual variation in the composition of the diets.

Three "anticholesterolemic" agents were employed. One contained soy bean oil (25 cc. daily) consisting of about 40% unsaturated fatty acids in an emulsion with vitamin B_6 and B_{12}; the second was a mixture of betaine, sorbitol, B_6, B_{12} and nicotinamide and the third was a combination of the first two with the same dosage of soy bean oil. A non-nutritive, methylcellulose placebo and an isocaloric saturated fatty acid preparation served as controls. Each of these agents was administered for four weeks; each drug period was preceded and followed by a similar control period during which either no medication, a placebo or the saturated fatty acid preparation was given. Body weight, food intake and physical activity remained relatively constant.

Fluctuations of the blood lipids were of similar magnitude for each patient during the control and medication periods.

The mean values for cholesterol, phospholipids and lipoproteins obtained from any drug period were not significantly different from those of the control periods. This study, then, failed to demonstrate significant alterations by any of the agents employed in the amounts used in any of the variables measured.

The Clinical Significance of Xanthelasma

By *Joseph B. Vacca, Goronwy O. Broun, Sr. and William A. Knight, Jr.* Department of Internal Medicine, St. Louis University School of Medicine, St. Louis.

Fifty-one persons with xanthelasma have been investigated with regard to a variety of clinical aspects. Sixteen were males, 35 females (including one Negress); 8 individuals had wasting diseases. Only one person had xanthoma elsewhere.

Most individuals had at least 3 total serum cholesterol determinations, usually on successive days or visits. Fifty-six percent of the males and 80% of the females had one or more cholesterols above 300 mg.%; 28% of the females, 25% of the males above 400 mg.%; 6% of the males and 9% of the females, above 500 mg.%.

Sixty percent of the females and 31% of the males were 20 pounds or more overweight. No correlation between degree of obesity and cholesterol could be determined; however, cholesterol levels were significantly lower in the debilitated group.

There was little, if any, correlation between age and cholesterol levels. There was, however, a rather definite correlation between cholesterol levels and size of the xanthelasma.

A striking variation in cholesterol levels was noted in many individuals. There seems to be a correlation between mean cholesterol level and variation in serial determinations.

69% of the males and 43% of the females had evidence of coronary artery disease. There were 10 and 4 individuals with peripheral or cerebral vascular disease, respectively. A family history of xanthoma or of prominent vascular disease was noted in only 6 and 8 individuals, respectively.

Of 35 patients tested, 27% of the males and 37% of the females had clinical or latent diabetes mellitus. Thirty-one of the females were postmenopausal, including 10 who were prematurely menopausal.

It is concluded that xanthelasma are not uncommon, occur mostly in obese, postmenopausal women and are usually associated with an elevated blood cholesterol and coronary artery disease. While xanthelasma may be manifestations of familial hypercholesterolemic xanthomatosis, significant numbers of individuals have other conditions (obesity, diabetes, menopause) often associated with increased blood lipids and atherogenesis[297].

COLLAGEN DISEASES

A Study of Lupus Erythematosus

By *Edward A. Jones and Robert E. Hodges.* Department of Medicine, University Hospitals, Iowa City.

Ideas about the cause of lupus erythematosus recently have centered around abnormalities of the blood proteins. Dameshek's "autoimmune theory" presents strong arguments favoring such ideas. Mellors and his co-workers demonstrated that gamma globulin was localized in the thickened capillary walls of the wire loop lesions of the kidney. This suggests that the generalized vasculitis in systemic lupus erythematosus may result from abnormal globulins. We have evaluated clinical and laboratory findings in the light of this hypothesis.

The records of 50 patients with systemic lupus erythematosus were examined and 35 of these patients were studied relative to course of their disease, results of therapy and serologic protein abnormalities. The records of patients who died were reviewed for correlation of clinical features and pathologic findings. Standard serologic tests for syphilis, serum proteins, A/G ratios, electrophoretic patterns and lupus precipitin tests were evaluated.

The most frequent symptoms and signs were weakness, arthralgia, arthritis, rash and fever. Involvement of multiple systems was common, though in many patients involvement of the kidneys was the most striking manifestation of the disease.

Examination of the laboratory tests revealed that 26% of the serologic tests for syphilis were anticomplementary and 21.4% were positive. The most consistant abnormality associated with the anticomplementary phenomenon was reversal of the A/G ratio. Over half of the patients whose sera were studied electrophoretically had significant elevation of the alpha 2 and gamma globulins. The L.E. precipitin test of Jones and Thompson, probably resulting from serum globulin fraction disproportion, was positive in 54.6% of proven lupus cases. In only 3 patients did the degree of globulin abnormality progress as the patient deteriorated. The order of reliability observed in tests for diagnosing lupus erythematosus was: L.E. cell phenomenon, L.E. precipitin test, globulin abnormalities and biologic false positive tests.

Therapy by several agents was compared in this series of patients. Steroids provided the most prompt symptomatic relief but did not seem to prolong life. Antimalarial drugs were less effective. Injections of concentrated leukocytes (Kurnick) provided symptomatic relief in 4 cases.

The Effect of Serotonin Antagonists on the Exaggerated Response to Serotonin in Patients with

Rheumatoid Arthritis and Related Diseases

By *Arthur L. Scherbel and John W. Harrison.* Department of Rheumatic Disease, Cleveland Clinic Foundation and Frank E. Bunts Educational Institute, Cleveland.

We have shown previously that intradermal or intra-articular administration of serotonin results in an exaggerated reaction in patients with rheumatoid arthritis and related diseases. The purpose of this report is to describe the effect of known serotonin antagonists on this reaction.

Forty-one patients with rheumatoid arthritis or related diseases and 16 control subjects were each given an extravascular injection of 0.1 mg. of serotonin. The injection was made into the skin of the dorsum of the hand or into a small joint. Patients with active rheumatoid arthritis and systemic lupus erythematosus demonstrated an exaggerated response to serotonin characterized by a rapid spread of erythema, swelling and cyanosis over the entire hand lasting from 1 to 8 hours. Control subjects showed only a mild localized reaction characterized by slight erythema and swelling adjacent to the site of injection without cyanosis.

Twenty-one patients with rheumatoid arthritis or lupus erythematosus were given a serotonin antagonist intravenously at the height of the reaction following the administration of serotonin. Six patients were given 0.9 mg. of Hydergine. Four of these patients had complete disappearance of cyanosis and two had partial disappearance. Eight patients were given 2.0 mg. of 2-brom-d-lysergic acid diethyl amide, and all patients responded by decrease in cyanosis; in 5 patients the disappearance was complete. Seven patients were given 2 mg. of 1-methyl-methergine-tartrate and cyanosis disappeared completely in 6 patients.

The exaggerated response to extravascular serotonin in these patients can be antagonized in vivo by various serotonin antagonists. Hydergine is the weakest serotonin antagonist and 1-methyl-methergine-tartrate is the most potent.

Analysis of the L.E. Cell Phenomenon in Rheumatoid Arthritis and Disseminated Lupus Erythematosus by Continuous Flow Electrophoresis

By *Eli M. Katz, Irving A. Friedman, Wilson H. Hartz and Charlotte Feldhake.* Hematology Laboratory, Hektoen Institute for Medical Research, Cook County Hospital, Chicago.

In a previous study, the L.E. cell phenomenon was found in 25 of 91 patients presenting the classic clinical picture of rheumatoid arthritis. In this study, the continuous flow electrophoretic apparatus was selected as a means to further this investigation.

Blood from 7 patients with disseminated lupus erythematosus (DLE) and from 5 patients with rheumatoid arthritis (RA) exhibiting the L.E. cell phenomenon was subjected to continuous flow electrophoresis. The globulin and albumin fractions obtained from this procedure were then dialyzed against pooled plasma or polyvinyl-pyrrolidone (PVP). Following the method of Haserick and Bortz, L.E. cell preparations were then made from the dialysates. Each L.E. cell preparation was arbitrarily graded as to intensity of L.E. cells, rosettes and nucleophagocytosis. Identification and quantitations of the dialysate fractions were carried out by horizontal filter paper electrophoresis.

L.E. cells were found with the gamma globulin fractions in 4 of the 7 (DLE) cases studied. Rosettes were found with the gamma globulin fractions of all 7 cases. No L.E. cells or rosettes were found with the albumin, alpha^1, alpha^2 or beta globulin fractions. Nucleophagocytosis (tart cells) was present with the whole serum and with the gamma globulin fractions in 6 cases. Tart cells were found with fractions other than the gamma globulin in 4 cases.

L.E. cells were found with the gamma globulin fraction in one of 5 cases of (RA) exhibiting the L.E. cell phenomenon. Rosettes and tart cells were found with the gamma gloublin fractions in all 5 cases. Tart cells were found with fractions other than the gamma globulin in two cases.

The factor responsible for the L.E. cell phenomenon, in both (DLE) and (RA), has been found to migrate with the gamma globulin component of the serum. In both (DLE) and (RA) nucleophagocytosis has been found to occur coincidental with typical L.E. cells.

The Effects of Prolonged Treatment with Large Doses of Prednisone on the Course and Prognosis of Severe Lupus Nephritis

By *Victor E. Pollak, Conrad L. Pirani, Robert C. Muehrcke and Robert M. Kark.* Departments

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Since treatment with adrenocorticosteroid hormones has improved the prognosis in systemic lupus erythematosus (SLE), most patients die of renal involvement—lupus nephritis. Clinical, biochemical and serial renal biopsy studies have been made on 65 patients with SLE. The effects of two therapeuatic regimens on the renal histology and course of severe lupus nephritis (lupus glomerulonephritis) were compared in 24 patients. Large doses of adrenocorticosteroid hormones were more effective than conventional doses.

Twelve patients with lupus glomerulonephritis, diagnosed by biopsy, received doses sufficient only to control their clinical symptoms (25 to 100 mg. cortisone). All have died (mean survival time 11.9 months). Six months after the biopsy only 5 were alive; 3 survived 18 months.

Subsequently, 12 patients with lupus glomerulonephritis were treated vigorously for 6 months with prednisone up to 60 mg. daily. All were made "Cushingoid." After 6 months the clinical, functional and histologic status of the kidney was reassessed, and the dosage was reduced to conventional levels only if the renal histology was improved. Two died within one month; one within four months of the first biopsy. The other 9 are alive, 7 for more than 18 months and 2 for more than 6 months after the biopsy. The mean follow-up time is 15.6 months. Only one patient has significant azotemia.

When first studied, both groups had equally severe glomerular lesions—with cellular proliferation, basement membrane thickening, local necrosis, karyorrhexis, fibrinoid and hematoxyphil bodies—features indicating severe and progressive disease. In the low dosage group these lesions progressed rapidly in most instances; improvement was not observed. In the high dosage group, serial studies showed thickening of the glomerular basement membrane but a decrease or disappearance of cellular proliferation, local necrosis, fibrinoid, karyorrhexis and hematoxyphil bodies —features indicating improvement and relative quiescence of the renal disease.

ENDOCRINES AND METABOLISM

The Effects of Cortisone and Vitamin B_{12} on Histochemical Changes in Liver and Heart of Starved Mice

By *Richard L. Davis and W. Lane Williams.* Department of Pathology, University of Minnesota, Minneapolis and Department of Anatomy, University of Mississippi, Jackson.

Starvation was used to establish basal conditions that would offset the effects of reduced food intake and gastric retention of food in mice given cortisone. Four groups of 50 mice with an initial body weight of 22-24 Gm. were starved for 3 days: one received cortisone; one received cortisone and vitamin B_{12}; one received B_{12}; and one group served as controls. Another group of 50 mice fed ad lib. were given cortisone. Cortisone (2.5 mg.) and vitamin B_{12} (2 μg.) were injected subcutaneously daily during the 3 days of starvation.

Glycogen was demonstrated by the periodic acid-Schiff's reagent technic. In mice receiving cortisone, both those starved and those fed ad lib., there were significant increases in the glycogen in hepatic parenchymal cytoplasm and in the myocardium. In starved mice not injected with cortisone there was no stainable glycogen in the liver or heart. The decrease in ribonucleic acid (RNA) in hepatic parenchymal cytoplasm as demonstrated by the basic dye-ribonuclease hydrolysis technic was approximately the same in all mice not receiving cortisone. Mature lymphocytes were decreased in lymphoid organs and the livers were fatty in all of the mice. In starved mice injected with cortisone the incidence of diffuse pneumonia was 69%, and of pericarditis, 17%; in those not receiving cortisone the incidence was 11% and 0, respectively.

The data demonstrate that in the absence of food intake cortisone increases weight loss but continues to exert profound glycopexic effects on the liver and myocardium. Starvation does not significantly accentuate the decrease in cytoplasmic RNA of liver cells in cortisone-injected

mice. Vitamin B_{12} retards weight loss but does not alter responses of the liver, heart and lymphoid organs to the glucocorticoid.

Tyrosine Deshalogenase Activity in Normal and Diseased Human Thyroid Tissues

By *Edward A. Carr, Jr., William H. Beierwaltes, Laurence L. Duncan, Norma R. Spafford and Roy A. Stambaugh.* Departments of Medicine and Pathology, University of Michigan Medical School.

Thyroid tyrosine deshalogenase activity is known to be increased in animals after thyroid-stimulating hormone (TSH) and absent in certain goitrous cretins. We compared its activity in several thyroid diseases.

Surgically removed human thyroids were immediately frozen at -60° C. and stored. Thawed 150 mg. slices and boiled controls were incubated with iodine^{131}-labeled and nonlabeled monoiodotyrosine in Krebs-Ringer phosphate containing nicotinamide, TPNH and thiouracil at pH 7.4 for 20 minutes at 37° C. Tyrosine-bound and liberated iodine were separated chromatographically and their relative amounts determined by counting in a well-type scintillation counter with pulse height selector. Slices immediately adjacent to those analyzed were examined histologically. Tests were performed at each of 5 substrate levels (20, 35, 51, 100, 200 mμmoles). Results for each tissue type represent mean substrate de-iodination (mμ moles/hour/150 mg. tissue) at each substrate level. More than 200 mμ moles did not further increase activity significantly.

"Normal" (histologically normal areas of 7 thyroids removed for cancer elsewhere in the gland) values were 42, 70, 74, 129, 176. Eight exophthalmic goiters gave significantly higher results (51, 92, 115, 175, 247). (Std. errors of differences: 7, 14, 19, 50, 69, respectively. P for combined differences < 0.01). Values for 5 Hashimoto's (40, 53, 78, 85, 179) and 9 colloid adenomatous (51, 74, 83, 100, 151) goiters appeared "normal." One nonspecific thyroiditis gave low values (23, 28, 24, 40, 28).

Six thyroid cancers gave low values (37, 46, 74, 62, 80) with considerable variation. The least differentiated showed no activity. Homogenate confirmed this. Another (papillary) gave lower values than any benign thyroid except thyroiditis.

Increased activity in thyrotoxicosis and "normal" activity, despite numerically reduced follicles, in Hashimoto's struma suggest increased TSH. Activity appears "normal" in benign nontoxic nodules and sometimes decreases when function is sacrificed to malignant growth.

Circulating Antibodies to Thyroglobulin: a Potential Mechanism in Multiple Thyroid Diseases

By *Robert M. Blizzard, George J. Hamwi, Thomas G. Skillman and Warren E. Wheeler.* Departments of Medicine and Pediatrics, Ohio State University, Columbus, Ohio.

Experimentally, thyroglobulin antibodies have been produced by the injection of homologous thyroglobulin in animals. Doniach and Roitt, by an agar precipitin technic, have demonstrated circulating antibodies to thyroglobulin in patients with Hashimoto's thyroiditis and spontaneous myxedema. Since antibody formation is the result of extrathyroidal escape of thyroglobulin, it was postulated that many other thyroid disorders would be associated with demonstrable thyroglobulin antibodies. The R.B.C. hemagglutination technic was found to be more sensitive for demonstrating antibodies as shown by the fact that only 5 of 48 sera, positive by the hemagglutination technic, gave positive precipitin reactions.

Accordingly, the sera of 175 consecutive patients with thyroid disease were examined for thyroglobulin antibodies by the R.B.C. hemagglutination method. Our findings show that thyroglobulin antibodies are present in a wide variety of thyroid disorders. The sera of 34 of 94 patients with thyrotoxicosis, 8 of 19 patients with nontoxic nodular goiter, 7 of 10 patients with spontaneous myxedema and 3 of 5 patients with chronic thyroiditis were positive for such antibodies. In addition, antibodies were found occasionally in subjects with subacute thyroiditis, athyrotic cretinism and mothers of cretins, but not in a limited number of subjects with acute thyroiditis, carcinoma of the thyroid, adolescent and colloid goiters. In contrast, only 8 of 219 control subjects had positive sera.

This study suggests that release of thyroglobulin secondary to thyroid injury or hyperplasia, regardless of etiology, is the sole requirement for autosensitisation. It is further suggested that thyroid autodestruction may serve as a contributing factor in the development of spontaneous myxedema, the occasional spontaneous re-

mission of thyrotoxicosis and rarely in athyrotic cretinism.

Cortisone in vivo or vitro, and thyroxin, triiodothyronine and thyrotropic hormone in vitro did not inhibit the agglutination.

Sera of patients with thyroid disease were also tested for globulin levels and thymol turbidity reactions. There was suggestive but imperfect correlation between hyperglobulinemia and antibody formation but no correlation with the results of thymol turbidity determinations.

Antibodies to penicillin were present in an appreciable number of sera that were positive for thyroglobulin antibodies, suggesting that these patients respond easily to many weak antigenic stimuli.

The Effect of Tolbutamide on Glucose and Nitrogen Metabolism in the Totally Depancreatized Dog

By *Henry L. Wildberger and Henry T. Ricketts.* Department of Medicine, University of Chicago, Chicago.

Contrary to the view that the presence of pancreatic beta cells is necessary to the action of tolbutamide is the finding previously reported by us of a reduction by tolbutamide of blood and urinary glucose in totally depancreatized dogs. These animals received small suboptimal amounts of insulin necessary to maintain life. The present experiments were undertaken to determine whether the lowering of blood and urinary glucose is attributable to potentiation of injected insulin.

Mongrel dogs were completely depancreatized, kept in metabolism cages and maintained throughout the following procedures on a constant diet and two small doses of crystalline insulin daily. Observations were carried out during alternating control and experimental periods, each of 2 weeks' duration. Addition of 0.25 Gm. of tolbutamide twice daily by mouth resulted in a significant decrease of blood sugar and urinary glucose as described previously, but nitrogen excretion was not affected. Addition of 2 to 4 units of protamine zinc insulin instead of tolbutamide produced a diminution of blood and urinary glucose comparable to that given by tolbutamide and a distinct fall in nitrogen excretion.

Since under these conditions tolbutamide does not cause nitrogen retention whereas extra insulin does, it is concluded that the oral drug does not act by potentiating the maintenance doses of injected insulin. It seems likely that the primary action of tolbutamide takes place in the liver, for whose functional integrity a certain minimum quantity of insulin is necessary.

The Effect of Phenethylbiguanide on Pyruvate Utilization in Man

By *John A. Moorhouse, Stefan S. Fajans and Jerome W. Conn.* Division of Endocrinology and Metabolism, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, Michigan.

Phenethylbiguanide (DBI) is a recently developed oral hypoglycemic agent. In an effort to elucidate its mode of action we have studied the effect of DBI on intermediary carbohydrate metabolism in man.

Blood levels of glucose, pyruvate and lactate were determined during glucose tolerance tests (GTT) and during pyruvate tolerance tests (PTT) in healthy and in diabetic subjects before and after administration of DBI. The PTT is a new procedure designed to assess directly removal of injected pyruvate from the blood. Ten Gm. of sodium pyruvate are given intravenously and blood samples are drawn at suitable intervals for 3 hours.

After administration of DBI blood glucose levels were not altered in the healthy subjects, while in the diabetic subjects these levels were significantly reduced. In the healthy subjects DBI caused no changes in the fasting levels of pyruvate and lactate and only small increases of these levels during the tolerance tests. In the diabetic subjects, DBI caused large increases in fasting levels of pyruvate and lactate and even greater increases of these levels during the tolerance tests.

Thus, the DBI-induced delay in pyruvate disposal from blood, while present in normal people, is of much greater intensity in diabetic subjects. Furthermore, comparison of the data from normal and diabetic subjects suggests that the hypoglycemic action of DBI may be quantitatively related to this effect on pyruvate disposal. The results are consistent with in vitro findings of others that DBI produces the Pasteur effect, that is, a block in oxidative reactions, an increase in anaerobic glycolysis and an increase in glucose uptake.

Metabolic Studies in Man of Chlorpropamide, a New Oral Hypoglycemic Agent

By *Kelly M. West, P. C. Johnson and Allen R. Hennes.* Department of Medicine, University of Oklahoma School of Medicine and V.A. Hospital.

Chlorpropamide is closely related chemically to tolbutamide. It is more potent than tolbutamide in some animals, including man. We investigated the factors responsible for the increased potency.

We found that radioactive chlorpropamide (S^{35}) had a half-time of 36 hours in diabetics and nondiabetics. Since the plasma radioactivity was nondialyzable, it appears that chlorpropamide is bound to protein, which may account for its slow excretion. Virtually all of the radioactivity administered orally appeared in the urine, suggesting that chlorpropamide is completely absorbed and that no significant extrarenal excretion occurs. The chromotographic R_f value was the same in serum and urine, suggesting that chlorpropamide appears unchanged in the urine.

Plasma levels of chlorpropamide averaged 11.4 mg. % 2 hours after oral administration of 1 Gm. to 7 subjects whose weights averaged 150 pounds, indicating that almost half of the drug was in the plasma. The rate of disappearance from plasma of nonradioactive chlorpropamide also suggested a half-time of about 36 hours. The hypoglycemic responses of 20 normal subjects were significantly greater 2 hours after chlorpropamide, 1 Gm., by mouth than after tolbutamide. Chlorpropamide, 250 mg., produced responses in 6 subjects 2 hours after ingestion, comparable to those after 500 mg. tolbutamide. Responses to chlorpropamide, 500 mg., and to tolbutamide, 1 Gm., were comparable. Since neither drug is appreciably metabolized or excreted as early as 2 hours after oral administration, it seems likely that slower excretion and degradation do not account entirely for the greater potency of chlorpropamide. Three normal subjects exhibited marked improvement of intravenous glucose tolerance after chlorpropamide, 1 Gm.

Apparently the marked difference in potency between tolbutamide and chlorpropamide in man is due to the fact that chlorpropamide is excreted very slowly, is not metabolized and it is probably more potent at comparable plasma levels.

Alternate Pathways of Glucose Metabolism in Man: Factors Influencing the Excretion of Ketopentose

By *Eugene M. Baker, Irvin C. Plough and Edwin L. Bierman.* U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons Army Hospital, Denver.

In an evaluation of the significance of alternate pathways of glucose metabolism, we have measured urinary excretion of ketopentose in normal men under a variety of conditions. The method, a modification of that of Futterman and Roe, primarily measured D and L-xylulose. D-xylulose is an intermediate in the pentose phosphate pathway, while the L isomer appears in the glucuronic acid pathway. L-xylulose, normally excreted at the rate of 4 mg./day, is vastly increased in essential pentosuria, where conversion of the L to the D form fails to take place.

Exercise produced a slight increase in ketopentose excretion, while the administration of triiodothyronine caused a 3-fold rise. Although dietary fat had no effect, carbohydrate, either as a supplement or exchanged isocalorically for fat, caused a moderate increase in ketopentose excretion. A 30 Gm. supplement of casein increased output to nearly 3 times the basal rate, but a nutritionally incomplete protein, gelatin, given in the same amount, had very little effect. In all these experiments the increased ketopentose in the urine was L-xylulose. That the other isomer, D-xylulose, can appear in the urine, has been demonstrated in other experiments with intravenous infusions of ribose and xylose.

Changes in carbohydrate metabolism via the alternate pathways certainly occurred in these experiments. Although the results may indicate increased activity of the pentose phosphate pathway as a cause of increased ketopentose excretion, it is more reasonable to look for the cause in increased catabolism via the glucuronic acid pathway because the extra ketopentose was L-xylulose.

Diabetic-Type Glucose Tolerance Test in a Patient with Renal Glycosuria

By *W. James Kuhl, Jr., Harry J. Miller and John A. McMillen.* Medical Service, V.A. Research Hospital and Department of Medicine, Northwestern University Medical School, Chicago.

An abnormal glucose tolerance test characterized by a normal or elevated fasting blood glucose, a peak blood glucose exceeding 160 mg.%, persistence of hyperglycemia beyond the second hour and a fall to hypoglycemic levels between the third and fifth hours has been suggested to be

one of the earliest manifestations of diabetes mellitus.

This type of glucose tolerance test was observed on two occasions during study of a 66-year-old male porter who was receiving x-ray therapy for an epidermoid carcinoma of the right tonsillar fossa. Renal glycosuria with loss of 12-15 Gm. of glucose daily and normal fasting blood sugar levels were present. Further study revealed less hyperglycemia during a glucose tolerance test and cortisol infusion. A glucose tolerance test and tolbutamide administration resulted in no improvement in the early hyperglycemia, but there was more marked hypoglycemia with symptoms at 4 hours. Continued administration of a 300 Gm. carbohydrate diet resulted in progressive improvement in the oral glucose tolerance tests with a return to a normal pattern after 6 weeks. Renal glycosuria continued with the loss of 1.5-6.0 Gm. of glucose daily.

The continued renal glycosuria after return to normal of the glucose tolerance test is more suggestive of a starvation-type carbohydrate intolerance than of early diabetes mellitus.

Correlation of Urinary 17-Ketosteroid and Pituitary Gonadotropin Titers in Certain Endocrine Syndromes

By *Raymond C. Mellinger and Richmond W. Smith, Jr.* Division of Endocrinology, Henry Ford Hospital, Detroit.

Knowledge of the ovarian dysfunction in virilized states not caused by ovarian tumors is incomplete. In the adrenogenital syndrome (AGS), adrenal androgens have been postulated to inhibit the release of pituitary gonadotropin, resulting in a state of hypogonadotropic hypogonadism. Investigations showing increased titers of certain urinary androgens in patients with the Stein-Leventhal syndrome (S-LS) have been interpreted to indicate that ovarian dysfunction in this disorder, like that in AGS, is secondary to excessive production of adrenal androgens.

The relationship of urinary 17-ketosteroids to urinary gonadotropin levels has been evaluated in 35 subjects grouped as follows: AGS, 3; Cushing's females, 9; Cushing's males, 3; S-LS, 15; idiopathic hirsutism, 3; normal females, 2. Gonadotropin assays were performed by the mouse uterine weight method. In all groups the effect on gonadotropin titers was evaluated after adrenalectomy or prolonged adrenocortical suppression with 6-methyl prednisolone had resulted in marked reduction in 17-ketosteroid excretion. Urinary gonadotropin titers did not correlate with 17-ketosteroid levels. Abnormally low urinary gonadotropin titers were observed in only 3 subjects, all with Cushing's syndrome, whose 17-ketosteroid levels ranged from 7 to 27 mg. per 24 hours. Decreasing 17-ketosteroid secretion by adrenalectomy or 6-methyl prednisolone administration did not alter gonadotropin levels in normals or in subjects with adrenogenital or Cushing's syndromes. In 4 of 6 subjects with S-LS receiving 6-methyl prednisolone, gonadotropin titers rose one dilution but remained within normal limits.

These results indicate that 17-ketosteroids of adrenocortical origin are not potent inhibitors of gonadotropin secretion and suggest that they do not affect ovarian function through the mechanism of pituitary suppression.

Detection of the Heterozygous Carrier in Galactosemia

By *David Yi-Yung Hsia, Irene Huang and Shirley G. Driscoll.* Genetic Clinic, Children's Memorial Hospital and Department of Pediatrics, Northwestern University Medical School, Chicago.

Galactosemia is a hereditary disorder of carbohydrate metabolism characterized by vomiting, diarrhea, jaundice, poor weight gain and malnutrition during early infancy. Isselbacher and his co-workers have shown that the accumulation of galactose in this disease is the result of a deficiency of Gal-1-P-uridyl transferase.

The frequent occurrence of the condition in siblings and among the offspring of consanguinous matings together with its equal distribution in both sexes suggest that galactosemia is probably transmitted by a single autosomal recessive gene. In this situation, one would expect the disease to occur only in persons with two abnormal genes, one from each parent. The heterozygous carriers would usually be free of symptoms, but careful biochemical studies might reveal minor departures from the norm.

Gal-1-P-uridyl transferase activity was measured by the method of Anderson, Kalckar, Kurahaski and Isselbacher in 5 galactosemic families. The results expressed as Units/Gm. Hgb. of Gal-1-P-uridyl transferase activity were as follows:

11	Normal Controls	4.5±0.47
12	Heterozygotes	3.3±0.36
8	Galactosemics	0.5±0.04

The differences between the means is significant at the 0.05 level, and about half of the levels of Gal-1-P-uridyl transferase among the heterozygotes lie below the range of normal controls.

While the results in a single individual must be interpreted with caution, the lower values of Gal-1-P-uridyl transferase found in a group of heterozygotes offer confirmation for the view that the lack of this enzyme is the primary biochemical lesion in galactosemia. Furthermore, the decrease of this enzyme in the heterozygote with one abnormal gene and its virtual absence in the homozygote with two abnormal genes extends the concept of being able to detect the heterozygous carriers biochemically in hereditary diseases.

The Mineral Content of Human Bone in Clinical Acidosis

By *James W. Agna, Lionel R. King and Harvey C. Knowles.* Metabolism Laboratory, University of Cincinnati College of Medicine, Cincinnati.

There is evidence in animals that a decrease in bone Na, CO_3 and possibly K occurs during acute acidosis. The amount of Na released was of a degree to suggest significant buffering action. Studies have been conducted to determine if similar changes occur in clinical acidosis in man.

Twenty-six specimens of skull, rib and ilium were obtained from 11 patients dying in acidosis of different causes with CO_2 content < 14 mM/L. A Na-retaining tendency was present in 7 instances. Thirty-seven specimens from 14 normal subjects who died suddenly served as controls. Cations were separated by column chromatography. Expressed per Kg. of fat-free solids, no differences could be shown between concentrations of water, N, Cl, K, Ca, P, CO_3 or Na. Nor were differences demonstrated between the concentrations of water, K and Cl expressed per organic phase (ref. N) or P expressed per crystalline phase (ref. CA). However, there was a mean significant decrease ($P < 0.02$) of 8 mM of CO_3 and probably significant decrease ($P < 0.05$) of 1.3 mM of Na expressed per M of CA between normal and acidotic skull, rib and ilium.

The findings suggest that changes may occur in acidosis. However, the decreases were considerably less than those reported in animals. Reasons for the disparity could be different degrees of acidosis, the complexity of disease processes in man interfering with the effects of acidosis or a more stable bone structure in man.

Intracellular Magnesium in Delirium Tremens and Uremia

By *William O. Smith, Richard J. Warren and James F. Hammarsten.* Medical and Radioisotope Services, Oklahoma City V.A. Hospital and Department of Medicine, University of Oklahoma School of Medicine, Oklahoma City.

Extracellular (serum) magnesium is often, but not always, low in patients with delirium tremens. It is usually elevated in patients with uremia. On the other hand, some patients have either an abnormally low or high serum magnesium without clinical symptoms. It has been postulated that the symptoms of magnesium deficiency or excess may depend rather on the intracellular concentration. We have measured intracellular (erythrocyte) magnesium in patients with delirium tremens and uremia by a new EDTA spectrophotometric titration method.

Twelve patients with delirium tremens had a plasma magnesium of 1.50 ± 0.28 mEq./L. compared to our normal of 1.80 ± 0.13 mEq./L., a 17% decrease in the mean ($P < .001$). However, 5 of the patients had values within the normal range (mean $\pm$ 2 S.D.). The erythrocyte magnesium was 3.19 ± 0.75 mEq./L. in this group compared to the normal of 5.29 ± 0.34 mEq./L., a 40% decrease in the mean ($P < .001$). All erythrocyte values were below the normal range. Electrocardiographic and electroencephalographic abnormalities were noted in some of these patients. Following 72 hrs. of therapy with intramuscular magnesium sulfate, the plasma magnesium increased to 2.17 ± 0.46 mEq./L. and the erythrocyte magnesium to 4.66 ± 0.48 mEq./L.

Fourteen patients with uremia and central nervous system depression had a plasma magnesium of 3.17 ± 1.30 mEq./L., a 76% elevation in the mean ($P < .001$). The erythrocyte magnesium was 8.84 ± 1.71 mEq./L., a 67% elevation ($P < .001$). However, 4 of these patients had a plasma value within the normal range; all erythrocyte values were elevated. Electrocardiographic abnormalities were noted in some of the patients where the elevated magnesium was the only electrolyte disturbance found.

Intracellular magnesium appears to correlate better with clinical symptomatology than does extracellular magnesium, particularly in magnesium deficiency.

The Effect of Exercise on the Difference Between the pH of Plasma and Whole Blood

By *S. Raymond Gambino.* Department of Pathology, St. Luke's Hospital, Milwaukee.

Recently Severinghaus and co-workers and Selenius have reported higher pH values for plasma than for whole blood, but these differences appeared to occur randomly and were largely unexplained.

We have measured the pH of whole blood and true plasma in the resting state in 9 normal subjects and 55 hospital patients and serial determinations after graded muscular activity in 6 subjects and 7 patients. The Beckman Model G pH meter and a Sanz micro-electrode thermostatically regulated at 38°C. were used with 3 National Bureau of Standards buffers (pH 4.025, 6.835 and 9.070 at 38°C.) as references. All 3 buffers were read before and after each series and were required to check within 0.01 units. The 6.835 buffer was read between each blood or plasma determination. The Sanz electrode and Beckman pH meter were checked against a McInnes-Belcher electrode and Cambridge Research Model pH meter and agreed with 0.01 units on the same samples of whole blood and plasma.

Most subjects and patients in the resting state showed plasma pH values 0.01-0.02 higher than those of whole blood. Patients with anoxia had higher differences between plasma and whole blood. Local muscular activity (hand exercise) or systemic exercise (step-ups or sprinting) caused the pH of whole blood to fall more than that of plasma, with differences up to 0.10 pH units. These differences were observed both in arterial and venous blood and were proportional to the duration and severity of exercise. They were unaffected by the speed of centrifugation or remixing and repeat centrifugation.

Preliminary studies show a correlation between the blood lactic acid concentration and the difference in pH between plasma and whole blood.

Serum Hexosamine and Acid Mucopolysaccharide Excretion in Hereditary Primary Systemic Amyloidosis

By *Charles E. Jackson and Walter D. Block.* Caylor-Nickel Clinic, Bluffton, Indiana and University of Michigan, Ann Arbor, Michigan.

An atypical protein peak between the alpha-2 and beta globulin area on free electrophoresis had been found by Block, Rukavina and Curtis (1956) to be associated with hereditary primary systemic amyloidosis. Subsequently it had been found in this family that this electrophoretic peak is not specific for amyloidosis. The present study represents an attempt at further characterizing the electrophoretic abnormality seen in this family in association with primary amyloidosis.

Electrophoretic analysis at ph 4.5 of sera from 10 individuals showing the atypical alpha-2 globulin in this family revealed mucoprotein peaks similar to those prealbumin peaks obtained in normal sera.

In 6 patients with clinical evidence of disease, the serum hexosamine levels (83-128 mg.%) by the Elson-Morgan technic did not vary consistently from normal.

In 5 patients the urinary acid mucopolysaccharide excretion on 3 consecutive days determined by the method of Di Ferrante and Rich averaged 2.1-6.8 mg. per day and did not differ significantly from a group of 6 normals (1.9-8.4 mg.).

By the technics used in these cases of hereditary primary systemic amyloidosis, no consistent aberration from normal could be detected in serum mucoproteins, serum hexosamines or urinary acid mucopolysaccharides.

GASTROINTESTINAL SYSTEM

Effects of Amine-Liberators on Gastric Secretions in the Rat

By *F. J. Owens and G. M. C. Masson.* Cleveland Clinic, Cleveland.

Acute hemorrhagic ulcers restricted to the glandular portion of the stomach can be readily elicited in rats by endogenous liberation of histamine and/or serotonin. Whether the amines involved act by increasing gastric acid secretions is the object of the present paper. This was investigated in groups of at least 6 adult rats each, in which the pylorus was ligated following fasting. Drugs were then injected; the animals were deprived of fluid and killed 6 hours later. Volume, pH, free and total acidities were measured. In

the controls the following mean values were obtained: volume, 12.6 ml.; pH, 1.2; free acidity, 71 degrees; and total acidity, 113 degrees. With histamine liberators (Polymyxin B, compound 48/80, dextran or ovomucoid) there were decreases in volume and acidity independent of the presence of ulcers. Thus, with Polymyxin B (1 mg./Kg.) the respective values were: 5.4 ml., 2.4, 21 degrees and 68 degrees. When promethazine (50 mg./Kg.) was given with Polymyxin B, volume and acidity were further depressed and ulcerations were prevented. With atropin (5 mg./Kg.) plus Polymyxin B the volume was reduced to 1.9 ml. with no detectable acidity, while ulcers were present in all the rats. The effects of 5-hydroxytryptophan (300 mg./Kg.) were in the same direction, but not as marked as those with histamine liberators. The respective mean values were 6.3 ml., 1.5, 54 degrees and 115 degrees; these were not significantly altered by simultaneous treatment with BOL (4 mg./Kg.), although ulcers were inhibited. Injections of histamine (30 mg., free base) and/or serotonin in oil (6 mg., free base) also reduced volume and free acidity; no ulcer was present, although serotonin produced severe renal cortical necrosis. In conclusion, amine liberators do not produce ulcerations in rats as a result of an increase in volume and acidity of gastric secretions. Probably their mechanism of action is primarily due to the effect of amines on the vessels of the stomach.

Serum Turbidity Following a Fat Meal as a Test of Malabsorption

By *J. D. Kabler, William H. Atwood, Jr. and Robert F. Schilling.* Department of Medicine, University of Wisconsin Medical School, Madison, Wisconsin.

This study was done to evaluate a simple test of fat absorption in subjects with and without gastrointestinal disease.

A fasting serum sample was drawn. The test meal contained 0.5 Gm. of butter per Kg. body weight, two pieces of toast, 100 cc. orange juice and a cup of black coffee. Only water was allowed during the test. Venous blood was drawn each hour thereafter for 5 hours. The change in optical density of each serum sample relative to the fasting serum was determined in a Beckman Model DU spectrophotometer at 620 mμ. An increase in optical density of 0.1 or greater in any post cibum serum sample was considered normal.

One hundred forty-six tests were done in 124 persons. In 49 subjects without evidence of gastrointestinal symptoms or disease, 59 tests were done. Six subjects had abnormally low increments of serum optical density following the test meal. Subsequent tests were normal in 3 of these subjects. Of 23 subjects with gastrointestinal disease having no evidence of steatorrhea, 2 had abnormally low serum optical density increments.

Twenty-five patients with various malabsorption syndromes (chronic pancreatitis, total common duct obstruction, extensive small bowel resection, regional enteritis and idiopathic steatorrhea) were tested by other technics including vitamin A tolerance tests, I^{131}-tagged triolein, vitamin B_{12} absorption and glucose tolerance test. Every one of these subjects with abnormal absorption had subnormal rises of optical density following the fat meal.

Additionally, abnormal test results were commonly found in the presence of thyrotoxicosis and advanced diffuse hepatic disease.

In the absence of thyrotoxicosis or hepatic disease, measurement of the increase in serum optical density following a standard fat meal is a useful, simple screening test for malabsorption of fat.

The Use of a Standardized Carotene-loading Test in the Diagnosis of Malabsorptive States

By *Paul R. Finley and Richard P. Doe.* Departments of Medicine and Pathology, V. A. Hospital and University of Minnesota Medical School, Minneapolis.

A simple but reliable screening test for the detection of malabsorptive states involves the administration of carotene in a standardized challenge dose. Because carotene absorption is closely parallel to the absorption of fat from the gastrointestinal tract, the observed rise of carotene in the plasma should reflect the extent of malabsorption present.

Carotene capsules (5,000 units) were given orally, 15,000 units t.i.d. for 3 days. Two fasting blood specimens were obtained, the first before carotene administration, the second 4 days later. Plasma was extracted with petroleum ether and read directly in a colorimeter. Subjects included 75 men in whom no malabsorption was apparent and 75 others who were afflicted with various malabsorptive states, including idiopathic sprue, chronic pancreatitis, carcinoma of the pancreas,

patients with total pancreatectomy, cirrhosis, patients with gastrectomies and short-circuit procedures, regional enteritis and ulcerative colitis. Ability to absorb fat was also tested by regulating the dietary fat and determining stool fat and nitrogen.

In 75 normal subjects, initial carotene levels ranged from 32γ% to 278γ% (mean, 108γ%). Response to the challange dose of carotene was manifested by elevation of the plasma carotene, measured on the 4th day, of not less than 45γ% nor more than 117γ% (mean, 63.4γ%). In many cases, the rise was double the initial value. In 75 patients with malabsorption, initial carotene levels ranged from 0γ% to 123γ% (mean, 41.8 γ%), but the response to the loading dose was never more than 39γ% and many times was less than 5γ% (mean, 15γ%).

Thus, overlap of normal and abnormal states often occurred when only fasting levels were considered, but absolutely no overlap was observed when the increment in plasma concentration in response to carotene loading was employed.

The utility, simplicity and sensitivity of a standardized carotene-loading test in the detection of the malabsorptive states make it a valuable adjunct in their diagnosis and assessment of treatment.

Vascular Responses in the Mucosa of the Human Colon

By *Jack D. Welsh.* Department of Medicine, University of Oklahoma School of Medicine, Oklahoma City.

Direct observations were made of the exposed colostomy mucosa in a patient with ulcerative colitis. Color changes and changes in the small mucosal vessels as observed through a dissecting microscope were correlated with variations in temperature of the exposed mucosa using a Stoll-Hardy radiometer as an indicator of directional changes in blood flow. Reference was also had to pulse rate and measurements of temperature variation over the dorsum of the hand. It was first established that color changes reflected alterations in caliber of the small vessels observable through the microscope, and these, in turn, corresponded closely to the temperature readings. During periods of relative inactivity of the bowel, lasting for periods of 2 to 3 hours, there occurred a rhythmic rise and fall of small amplitude in the colon temperature. These wave-like changes usually correlated inversely with the temperature of the dorsum of the hand. When vigorous motor activity of the colon occurred there was an associated sustained increase in mucosal temperature and color. Activity usually increased following a meal, but the colon temperature and color increased predictably and the pulse rate increased uniformly after eating, returning to base line levels after approximately 80 to 120 minutes. Again skin temperature responded inversely with an early fall and a late rise. Stressful stimuli in the form of discussions of emotionally charged topics induced a marked hyperemia with an associated increase in colonic temperature. Parenteral exhibition of anticholinergic agents such as tridihexethyl iodide (Pathilon), on the other hand, inhibited motor activity, decreased colonic temperature and brought about a relative pallor of the membrane. This effect was sufficiently potent to block the expected postprandial mucosal hyperemia with its rise in colon temperature. In 18 of 21 experiments involving all of these maneuvers, a roughly inverse relationship between the temperature of the colon and that of the dorsum of the hand was evident to some degree. These data appear to support the inference that a reciprocal relationship may exist between cutaneous and visceral blood flow, an effect which has long been suspected but has not been satisfactorily demonstrated experimentally.

INFECTIOUS DISEASES

The Inhibition of Streptococcal Diphosphopyridine Nucleotidase by Sera from Patients with Rheumatic Fever and Patients with Uncomplicated Streptococcal Pharyngitis

By *Gerson C. Bernhard and Gene H. Stollerman.* Department of Medicine, Northwestern University Medical School, Chicago.

Diphosphopyridine nucleotidase (DPNase) has recently been identified in cardiotoxic fractions of culture supernates of group A streptococci. A study was undertaken to determine the behavior of serum anti-DPNase activity (ASDA) in patients with uncomplicated streptococcal infections and in patients with acute and quiescent rheumatic fever compared with three known streptococcal antibodies.

ASDA activity in serum was measured by minor modifications of the spectrophotometric methods recently developed by Kellner and Bernheimer. Replicate determinations were made on several pools of human sera and showed standard deviations that were within 10% of the mean titers. ASDA activity was sharply localized to the gamma globulin fraction of human serum and the inhibition was directed specifically against streptococcal DPNase and not against DPNase derived from other species.

Of 87 children with pharyngitis associated with a positive culture for group A streptococci, a significant rise in ASDA titer occurred in 63%; in antistreptolysin O (ASD) titer, in 62%. Determinations of antihyaluronidase (AH) and antistreptokinase (ASK) on these sera are in progress.

Of 30 patients with acute rheumatic fever, elevated titers of ASDA occurred in 87% and of ASO in 88%. AH titers determined in 15 patients were elevated in 60%.

A group of 18 ambulatory rheumatic patients were studied who were shown to be free of streptococcal infection for at least one year by negative throat culture and the absence of an increase in the titer of ASO, AH and ASK antibodies determined bi-monthly during this time. ASDA titers were also found to be uniformly low in this group.

ASDA activity in human sera parallels closely the behavior of known streptococcal antibodies in patients with uncomplicated streptococcal infection and in those who develop rheumatic fever. It reflects streptococcal infection about as well as the ASO titer and is measured with ease and accuracy.

The Effect of Buccally-given Streptokinase on Serum Complement

By *Irving Innerfield and Thomas Gilmore.* Enzyme Research Laboratory, Department of Medicine, New York Medical College-Metropolitan Medical Center, New York City.

Significantly increased serum complement activity was observed in 16 out of 18 patients on buccally-given streptokinase (SK). Pretreatment control serum complement levels ranged from 1.3–2.2 units, average 1.65 units. Following 3 weeks of uninterrupted buccal SK therapy (10,000 units every 4 hrs.) the complement levels ranged from 2.3–5.2 units, average 4.1 units.

Parenterally-given SK to rabbits and guinea pigs did not alter serum complement. In vitro, SK rapidly inactivated human complement but had no effect upon rabbit or guinea pig complement. Neither SK nor trypsin alone caused increased hemolysis of sensitized red cells.

Decreased dermal sensitivity to tuberculin developed in 4 out of 5 previously tuberculin-sensitive individuals on buccal SK therapy.

It is postulated that buccally-given SK accelerates the conversion of a plasminogen-containing complement constituent, (probably C'1) into a factor with complement-like activity.

KIDNEY

The Functional Status of the Denervated Kidney Following Successful Homotransplantation in Identical Twins

By *H. Earl Ginn.* Medical Service, V. A. Hospital, Oklahoma City and Department of Medicine, University of Oklahoma School of Medicine, Oklahoma City.

Bricker et al., in a study of renal function in a successfully transplanted kidney, found normal function except for failure of sodium excretion to respond normally to acute alterations of ECFV (extracellular fluid volume). They attributed the defect to denervation of a normal volume control system. The present studies were performed following successful homotransplantation in which the transplanted kidney was ischemic for a shorter period and bilateral nephrectomy was performed earlier than in the previous study.

Effective renal plasma flow and glomerular filtration rate were found normal for a single kidney. The ability to acidify and alkalinize urine in response to ammonium chloride and diamox, and to concentrate and dilute urine, measured by osmotic urine to plasma ratios, were normal. The kidney displayed normal diurnal variation in water and sodium excretion. During periods of sustained mental concentration, urinary output increased while PAH, inulin and creatinine clearances decreased comparably with results in patients with normally innervated kidneys. When orthostatic hypotension was induced, there was slight decrease in inulin and PAH clearances. The response to acute changes in ECFV was normal. During rapid infusion of hypotonic saline sodium excretion, free water clearance and osmolar clearance increased. Following isotonic saline infusion in the hydropenic state there was increase in sodium excretion and osmolar clearance with elaboration of hypertonic urine. Elaboration of hypotonic urine with increase in free water clearance and osmolar clearance followed rapid infusion of isotonic saline in the prehydrated state. During rapid infusion of hyperoncotic salt, poor albumin osmotic U/P ratios increased with decrease in water and solute excretion.

It is concluded that renal nerves are not necessary for chronic regulation of ECFV, for regulation of acute changes of ECFV or for a normal response of the kidney in the diversified functions tested, and, therefore, that the transplanted kidney may maintain normal existence.

Electronmicroscopic and Ultramicrobiochemical Studies of the Potassium-depleted Kidney

By *Robert C. Muehrcke and Sjoerd L. Bonting.* Departments of Medicine and Biochemistry, University of Illinois College of Medicine and Departments of Medicine, Presbyterian-St. Luke's Hospital, Cook County Hospital and Research and Educational Hospitals.

The purpose of this study is to investigate the defects of the renal tubules in potassium-depleted rats, using electronmicroscopic and ultramicrobiochemical technics.

Sprague-Dawley rats were depleted of potassium by potassium-free diet, cation exchange resins and sodium bicarbonate drinking solutions. Kidney tissue was obtained at the height of potassium depletion and 10 days after slow potassium repletion.

Electronmicroscopic studies revealed marked changes limited to the mitochondria in the collecting tubules of the distal papillae. The earliest structural changes were mitochondrial swelling and fragmentation with release of osmophilic material into the cytoplasm. Ten days after potassium repletion, the mitochondria appeared normal.

Alkaline phosphatase (AP) and lactic dehydrogenase (LDH) activity was determined quantitatively by ultramicrobiochemical technics. Determinations of enzyme activity were made of normal and potassium-depleted kidneys. Decreased AP activity was found in the vessels, glomeruli, proximal tubules, inner medullary zone tubules and papillae base. Normal activity was observed in the proximal tubules and collecting tubules of the papillae tip. LDH activity was decreased in the glomeruli, cortical proximal tubules and papillae. Normal activity was noted in the outer medullary zone proximal tubules and blood vessels. There was increased LDH activity in the tubules of the IMZ.

These data indicate: (1) potassium depletion produces changes in enzyme activity which differ along the individual anatomic units of the nephron; (2) changes in the enzyme activity occurred before electronmicroscopic changes

were observed; (3) the mitochondrial degeneration of the collecting tubules seen with the electronmicroscope are the eosinophilic granules seen under the light microscope; (4) increased LDH activity in the IMZ with normal AP activity in the distal tubules and collecting tubules of the papilla tip may reflect the potassium-conserving mechanism of the potassium-depleted kidney.

Asymptomatic Persistent Proteinuria: Studies by Renal Biopsy

By *Victor E. Pollak, Conrad L. Pirani, Robert C. Muehrcke and Robert M. Kark.* Departments of Medicine, Presbyterian-St. Luke's Hospital, Cook County Hospital and Research and Educational Hospitals and Departments of Medicine and Pathology, University of Illinois College of Medicine, Chicago.

Proteinuria has been known to be a hallmark of serious renal disease for 120 years. Until recently, accurate diagnoses could not be made in asymptomatic patients in whose urine protein was persistently found on routine examination. In the last 3 years percutaneous renal biopsy has enabled us to investigate the renal histology in such patients during life.

Nineteen patients were studied. Proteinuria was found on routine examination for employment, insurance or admission to hospital for surgical conditions. No patient had a past history or physical findings suggestive of renal disease. Tests of renal function were normal except in 2 patients with very mild azotemia.

The renal biopsies of 18 patients were abnormal. Pyelonephritis, "lipoid nephrosis" and arteriosclerosis were each found in one instance; glomerulonephritis of varying histologic types in the other 15. The severity of the lesions was graded as moderate to considerable in 6, and mild or minimal in 9. Glomerular basement membrane thickening was the outstanding feature in 5. Four others had basement membrane thickening and proliferative lesions. In all 6 cases classified as having mild proliferative changes only a proportion of the glomeruli were involved. Although all were well and no patients had evidence of recent infection, polymorphonuclear leukocytes were found in the glomeruli, in addition to epithelial cell proliferation, adhesions and crescent formation—suggesting that there was active glomerulonephritis.

These preliminary observations indicate that the renal disease underlying asymptomatic persistent proteinuria can be diagnosed by renal biopsy in most patients. The severity and activity of the lesion can be assessed. With severe lesions the prognosis is relatively grave; one such patient has already died. With milder lesions which show evidence of activity, the natural history can only be assessed by long-term studies. To date, all these patients have continuing proteinuria.

Studies on Gamma Globulin Deposition in the Human Kidney in Health and Disease

By *John H. Peters, Philip Freedman and Robert M. Kark.* Departments of Medicine, University of Illinois College of Medicine, Presbyterian-St. Luke's Hospital, Cook County Hospital and Research and Educational Hospitals, Chicago.

Fluorescent antibody technics (Coons et al.) were used to study the role played by immune mechanisms in renal diseases.

Renal tissue was obtained from patients by needle biopsy, immediately frozen in liquid nitrogen (−180° C.), cut into 6 micron sections in a cryostat at −20° C., and then stained for 45 minutes with horse antihuman gamma globulin (conjugated with fluorescein isocyanate or rhodamine 200) and/or rabbit anti-guinea pig complement (conjugated with rhodamine 200) and studied with an ultraviolet light microscope. The fluorescein imparted apple-green fluorescence and the rhodamine an orange fluorescence to the material conjugated. Adjacent serial sections were used as controls to demonstrate specificity of the stain. Blocking of specific fluorescence was accomplished by first staining the tissue with unconjugated anti-gamma globulin, and when sections were stained using conjugated antisheep gamma globulin, no specific localization of the fluorescent material occurred. Serial sections were also studied by conventional staining technics.

Specific fluorescent staining of the glomerular basement membrane was seen in cases of glomerulonephritis, systemic lupus erythematosus and polyarteritis nodosa. Less intense basement membrane staining was found in cases of scleroderma and sickle cell trait with hematuria. In one case of diabetic glomerulosclerosis patches of specific glomerular and tubular basement membrane fluorescence was seen. Specific fluorescent staining was also observed in the small blood vessels and arterioles in glomerulonephritis,

scleroderma and polyarteritis nodosa. Localization was mainly intimal and subintimal, occasionally medial. Specific fluorescence was occasionally seen in tubular cells.

Thus far, renal tissue from patients with membranous glomerulonephritis have not stained positively with anticomplement.

Specific localization of the fluorescent stains was not seen in renal tissue from healthy individuals, patients with rheumatoid arthritis, rabbits or rats.

By treating the slides with citrate buffer at pH 3.45, specific fluorescence was decreased, indicating elution of the gamma globulin. When eluates from these reactions were concentrated and electrophoresis run, a thin band was found to migrate in the area of gamma globulin.

It is concluded from these observations that an immune mechanism(s) may play a prominent role in those renal diseases in which gamma globulin has been demonstrated in areas of tissue damage.

Hypotension during Hemodialysis: Its Prevention Using Human Serum Albumin

By *Frank T. Maher, James C. Broadbent, John A. Callahan and Guy W. Daugherty.* Mayo Clinic and Mayo Foundation, Rochester, Minnesota.

The purpose of this study was to determine the effectiveness of human serum albumin in preventing the occurrence of hypotension during hemodialysis. By means of the Skeggs-Leonards apparatus, hemodialysis was performed 56 times for 28 patients. For 33 dialyses for 22 patients, the blood path of the apparatus was filled with whole blood; for 23 dialyses for 6 patients, it was filled with a 5% solution of human serum albumin in a 0.9% solution of sodium chloride. Since hypotensive reactions occur during the first hour of dialysis and frequently within the first 15 minutes, the 2 groups were compared on the basis of blood-pressure recordings made during the first hour of dialysis. In 21 of the 33 dialyses in which blood was the priming solution, the blood pressure did not change significantly. In 6 of the 21, there was a slight decrease in pressure, averaging 11 (6-17)% of the control systolic pressure and 15 (0-36)% of the control diastolic pressure. In 12 of the 33 dialyses, a pronounced decrease in pressure occurred, averaging 43 (33-65)% of the control systolic pressure and 36 (15-63)% of the control diastolic pressure. In 11 of the 23 dialyses in which human serum albumin was the priming solution, the blood pressure did not decrease during the first hour. In 12, a slight decline was present, averaging 10 (5-22)% of the control systolic pressure and 9 (0-28)% of the control diastolic pressure. Thus, the hypotensive reaction that may characterize the first hour of hemodialysis appears to be related to use of whole blood, for such a reaction was not observed when a 5% solution of human serum albumin was used in place of whole blood to fill the dialyzer assembly.

Bioassay of Aldosterone before and after the Inducement of Renoprival Hypertension

By *Milam S. Cotten and H. G. Langford.* Department of Medicine, University of Mississippi School of Medicine, Jackson, Mississippi.

The purpose of this experiment was to determine whether a direct relationship exists between the level of plasma aldosterone and the development of hypertension in renoprival dogs.

The control blood pressure and control (normotensive) plasma aldosterone was determined in 6 dogs. The blood pressure was determined in the unanesthetized stage by direct femoral arterial puncture with a No. 19 gage needle connected to a mercury manometer. The plasma aldosterone was determined in DOCA equivalents (measured by change in Na/K ratio in the urine) by use of bilaterally adrenalectomized rats as the biologic system by the method of Llaurado.

Renoprival hypertension was induced and the change in blood pressure and plasma aldosterone was determined at 3 days.

Three of the six dogs had a significant increase in blood pressure (greater than 30 mm. Hg). Aldosterone was not detectable in the control plasma of 5 of the 6 dogs, a level of 0.06 μg.% being present in the sixth. Three days after removal of the last kidney, aldosterone was detectable in the plasma of 3 of the dogs, 0.05, 0.28 and 0.12 μg.% being present. There was no consistent relationship between the degree of hypertension and the plasma level of aldosterone. The dog with the greatest increase in aldosterone did not become hypertensive, whereas the dog with the greatest increase in blood pressure showed no increase in plasma aldosterone.

We conclude that the development of renoprival hypertension is independent of the plasma level of aldosterone.

NERVOUS SYSTEM

A Comparison of the Psychotomimetic Effects of Scopolamine, Lysergic Acid Diethylamide and N-ethyl-3-piperidyl Benzylate (JB 318)

By *Adrian M. Ostfeld, Harold M. Visotsky and Binyamin Z. Lebovits.* Departments of Preventive Medicine and Psychiatry, Research and Educational Hospitals, University of Illinois College of Medicine, Chicago.

This study was undertaken to examine the psychotropic effects of these agents and to compare them. Ten medical students received orally administered scopolamine in doses of .5 to 4.5 mg., 25 to 100 μg. LSD and 5 to 20 mg. JB 318. Doses of each agent were randomly administered in double-blind fashion. Effects on mood, thought and behavior were assessed by the subjects and a psychiatrist, a psychologist and an internist. The technics used to assess these effects were tape-recorded serial interviews, standard symptom questionnaire, Clyde Mood Scale and two psychological tests, the Rorschach and the Minnesota Multiphasic Personality Inventory (MMPI), which were administered before, during and after the period of drug effect.

The most predictable psychotomimetic effect was the induction of illusions. "ED_{50}" for this effect was 3 mg. scopolamine, 100 μg. LSD and 10 mg. JB 318. At this dose, LSD induced visual hallucinations in but one subject. Anxiety, suspiciousness, hyperactivity, deep tendon hyperreflexia and slight diminution in recent recall predictably occurred. Ten mg. of JB 318 induced visual and auditory hallucinations in 5 subjects. Anxiety, suspiciousness and hyperreflexia also occurred, but to a lesser degree than following LSD. Drowsiness and diminished recent recall were more pronounced than after LSD. Three mg. of scopolamine induced somnolence or sleep. Confusion and disorientation were prominent, with visual hallucinations occurring in 3 subjects.

The changes in psychological test performance after LSD were compatible with an increase in isolation, fantasy and bizarre ideation. The same trends were present but to a lesser extent following JB 318. After scopolamine, there was a marked reduction in attention span and recent recall which made for inadequate test performance. It is concluded that toxic psychoses induced by varying agents overlap in some respects, but that characteristic alterations in mood, thought and behavior are induced by structurally different compounds.

Some Effects of Quiactin on Normal Behavior

By *Alfred B. Kristofferson and Robert H. Cormack.* Department of Psychology, University of Cincinnati.

Two experiments are reported concerning the effects of moderate doses of Quiactin on 6 behavioral variables involved in complex acts such as driving. The behavior measures are similar to those employed by Marquis et al. and include: (1) steadiness, or total time off target in a Whipple-type apparatus; (2) rate of tapping during a 5-minute period; (3) accuracy of steering, slow speed; (4) accuracy of steering, fast speed; (5) brake reaction time, slow speed, and (6) brake reaction time, fast speed. The last 4 measures were made with the A.A.A. Autotrainer in two 15-minute periods.

Experiment I used 16 subjects in a double-blind design, with a single 800 mg. dose of Quiactin versus placebo. Each subject served as his own control, counterbalanced, and tests began 50 minutes after taking the tablets. Four conditions comprised Experiment II: (1) no treatment; (2) 2 oz., 100 proof bourbon whiskey; (3) 400 mg. Quiactin 4 times a day for 2 days, last dose 1 hour before testing; and (4) placebo control on condition(3). Each of the 24 possible sequences of the 4 conditions was assigned at random to 1 subject.

No significant differences were obtained in Experiment I, and no trend was found in the pattern of differences. In Experiment II, of the 6 scores, 4 were "poorer" for alcohol than no treatment, 1 poorer for drug than no treatment and 1 poorer for alcohol than placebo, at a probability level less than .05. As in Experiment I, no trend appeared in the differences between drug and placebo and no difference approached significance.

Under the conditions described herein, performance is impaired no more by Quiactin than by placebo, while alcohol produces greater impairment than both.

PHARMACOLOGY

The Uricosuric Effect of Certain Oral Anticoagulant Drugs

By *George R. Thompson, William M. Mikkelsen and Park W. Willis, III.* University of Michigan, Ann Arbor, Michigan.

The anticoagulant drugs, ethyl biscoumacetate (Tromexan) and bishydroxycoumarin (Dicumarol), have recently been shown to have uricosuric properties. Salicylates have long been known to have both anticoagulant and uricosuric effects.

The uricosuric action of single oral doses of 2 short-acting coumarin preparations (ethyl biscoumacetate, 2000 mg. and acenocoumarin, 20 mg.) and of 2 short-acting indandione preparations (anisindione, 300 mg. and phenylindandione, 20 mg.) was measured in 4 healthy male subjects on a constant diet and was compared to the effect of a single oral dose of probenecid, 1500 mg. Urine and serum uric acid levels were determined both by the method of Block and Geib and by the uricase method; the results with these 2 methods agreed within 0.1 mg.% in each instance.

Each drug, in the dose given, had a similar effect on the aspects of blood coagulation measured (prothrombin, one- and two-stage methods, Factor VII, Factor V, clotting time and thromboplastin generation time) and uniformly resulted in reduction of the Quick one-stage prothrombin concentration to between 40 and 50%. A rise in urate clearance of over 50% with a fall of serum uric acid of over 25% was considered evidence of a uricosuric effect. By these criteria ethyl biscoumacetate (342% and 50%), probenecid 234% and 47%) and phenylindandione (88% and 30%) were found to have a uricosuric action, while anisindione (22% and 15%) and acenocoumarin (34% and 3%) lacked this effect in the doses employed.

RESEARCH METHODS

Observations on the Law of Initial Values in Therapeutic Research: a Study of the Effects of Diuretic Agents

By *David C. Mock, Adrian Kyriakopoulos, Mervin Clark, Edward N. Brandt and James A. Hagans.* Experimental Therapeutic Unit and Department of Medicine, University of Oklahoma School of Medicine and Medical Service, V.A. Hospital, Oklahoma City.

In 1931, Wilder, recognizing that the effects of a stimulus may depend on the functional state of the organ system at the time of stimulation, postulated a "law of initial values."

A possible application of the law was observed when 101 healthy, hospitalized subjects were tested for their response to diuretics. During two control days a regimen of constant composition of electrolytes, calories and water was established and maintained throughout the study. Daily 24-hour urine collections were made. On the third and fourth days the subjects were given in a double-blind fashion, either meralluride, acetazolamide, mercaptomerin or a placebo. Measurements were continued on the fifth day, but no agent was given.

All agents produced a diuresis significantly greater than the placebo (variance analysis: $P < .05$). The variation in response to diuretics, however, was striking. Following meralluride, for example, urinary response ranged from 9% decrease to a 98% increase. Arrangement of the data according to urinary excretion during the control period, however, produced a very consistent pattern. The 5 subjects whose urine output on the second control day was less than 75% of water intake increased their excretion 36 to 98% (mean 60.4%) after the diuretic. The 8 who excreted from 75 to 95% of ingested water on the second control day increased their excretion 0 to 38% (mean 16%), while the 6 whose control excretions exceeded 95% of fluid intake increased only 1.67%. Some subjects in this group actually decreased urine excretion after the diuretic (range: −9% to +14%). Differences between means of the 3 groups were significant at the .01 level.

Use of the regression function ($y = a + bx$) and the placebo data permitted the prediction of the Day 3 urine excretion from any Day 2 initial volume where no diuretic given. The difference between such predicted values and the observed

response in the diuretic data provided a much less variable and more accurate reflection of diuretic activity. It is likely that Wilder's "law" will have wide application to biologic data, especially in experimental therapeutics.

Measurement of Blood Flow in Minute Volumes of Specific Tissues in Humans

By *James F. Schieve and Richard W. Stow.* Department of Medicine, College of Medicine, Ohio State University, Columbus, Ohio.

Blood flow through a 4 to 5 mm.^{3} portion of tissue can be determined by measuring the rate of temperature change in the tissue before and after local arterial occlusion.

Before occlusion the rate of change of heat content ($\dot{T}_t$) of tissue equals the rate at which heat is brought to it by the arterial blood and produced by local metabolism, minus the rate at which heat is lost through venous blood drainage and net conduction to the surroundings. After occlusion the factors of arterial and venous blood flow are eliminated and a new rate of change ($\dot{T}_t'$) exists. The difference between these two rates of change ($\dot{T}_t$-$\dot{T}_t'$) equals the rate at which heat was brought to and taken from the tissue by the blood. If the temperature (T_a) of the local arterial blood, that (T_v) of the local venous blood and the thermal capacities of the tissue (C_t) and blood (C_b) are known, then by the formula $F = \frac{C_t}{C_b} \frac{(\dot{T}_t - \dot{T}_t')}{(T_a - T_v)}$, blood flow (F) may be calculated.

If it is assumed that T_v is the same as the local tissue temperature, all the factors for determining F are known except T_a. However, with an additional modified occlusion observation, the needed value T_a may be calculated.

The thermometer (sensitivity 4×10^{-4}°C. and response time 95% equilibrium in 0.27 sec.) and heating unit are housed in a 25-gauge needle. Femoral arterial occlusion was performed manually.

Twenty-five observations of skin blood flow were made in 3 subjects with the combined thermometer and heating unit placed 1-2 mm. beneath the skin surface of the right calf. Flows ranged from 1.3×10^{-4} to 82×10^{-4} Gm. of blood per Gm. of skin tissue per second.

An extention of the technic has led to the measurement of the temperature coefficient of flow, when flow is influenced by very local temperature changes. In 9 observations a value of +39%/°C. was found.

Determination of Blood Flow by Externally Placed Scintillation Detectors

By *Gunnar Sevelius and Philip C. Johnson.* Radioisotope Service, V.A. Hospital and Department of Medicine University of Oklahoma, Oklahoma City.

All current methods for estimating peripheral organ blood flow require either arterial catheterization or catheterization of the venous system efferent from the organ. The single exception is the kidney where one must assume a normal tubular function. The present report concerns the development of a new method which allows the estimation of organ blood flow without the necessity of catheterization. The method employs the intravenous injection of radioiodinated serum albumin and scintillation counting over the organ's and heart's surface projections. The following mathematically derived equation is used, which determines the fraction of the cardiac minute volume going to the organ:

$$\frac{I_o}{I} = \frac{A_o^2 \cdot T_h \cdot B_h^2}{A_h^2 \cdot T_o \cdot B_o^2}$$

where o = organ; h = heart; I = injected radioactivity; A = Area of time activity curve; T = time for radioactivity to pass the detector first time; B = equilibrium value of radioactivity as seen by the detector over the surface projection of each organ. By determining this fraction and applying it to the formula proposed by Prinzmetal for estimating cardiac minute volume, one can obtain organ blood flow. Data were obtained on both coronary and renal blood flows. These data obtained from normal subjects are in close agreement with published reports based on data obtained at catheterization. The validity of this new method for the kidney has been checked by performing simultaneous PAH clearances. The mean value obtained from 9 subjects of similar height and weight for this technic was 1087 ± 44 cc./min. (± S.E.) and the mean for PAH was 1059 ± 70 cc./min. with a mean individual difference of +4.7 ± 2.3%.

RESPIRATORY SYSTEM

A Simple Bedside Test of Respiratory Function

By *John P. Stevens, Thomas H. Snider and Benjamin M. Lewis.* V.A. Hospital, Dearborn, Michigan and Department of Medicine, Wayne State University College of Medicine, Detroit.

It is frequently desirable to assess whether significant airway obstruction exists while conducting a physical examination. Two simple tests of airway obstruction are the maximum breathing capacity and the timed vital capacity. Both, however, require special equipment not always available at the bedside. We have correlated the ability of a patient to blow out a standard book match held 6 inches from the open mouth with these tests of pulmonary function. Eighty % of 52 patients who could not blow out the match had maximum breathing capacities below 60 L. per minute, while 80% of 74 who could blow out the match had maximum breathing capacities above 60 L. per minute. Eighty-five % of the patients who could not blow out the match had a 1 second vital capacity below 1.60 L., while 85% of the 74 patients who could blow out the match had a 1 second vital capacity above 1.60 L.

We feel that this simple bedside test is a fairly reliable guide to airway obstruction. It may fail in patients with greatly reduced total vital capacity due to interstitial fibrosis, without airway obstruction, since in these patients the 1 second vital capacity may be a normal percentage of the reduced total vital capacity and still inadequate to extinguish the match. Even in these cases it would serve as a guide that further function studies should be obtained.

In conclusion, we have demonstrated a simple bedside screening test for some phases of pulmonary dysfunction which if abnormal should cause one to obtain the more accurate tests which are available.

Studies on the Effects of Sympathicoamines in Bronchial Asthma

By *Gordon L. Snider, Murray C. Arkin, Bernard H. Miller and Milton M. Mosko.* Chest and Allergy Departments, Michael Reese Hospital, Chicago.

This study was undertaken in an attempt to elucidate some of the factors which cause bronchial obstruction in asthma and to study the mode of action of sympathicoamines in this condition. Serial determinations of the maximum breathing capacity were used as a measure of airway obstruction in the following studies:

1. *The effects of repeated small doses of epinephrine subcutaneously.* In 24 of 45 studies, there was a significant response to the first injection of 0.3 mg. of epinephrine subcutaneously. There was an additional response to subsequent injections of 0.2 mg. of epinephrine in 10 of these 24 studies. In 8 studies there was no significant rise after a first injection. Thirteen studies showed no response. A cumulative effect of repeated small doses of epinephrine without troublesome side effects was thus demonstrated in 32 of 45 studies.

2. *The effects of sympathicoamine aerosols (epinephrine or isoproterenol) following a maximally effective dose of epinephrine subcutaneously and the effects of epinephrine subcutaneously after a maximally effective dose of sympathicoamine aerosol.* In 10 of 34 studies aerolized sympathicoamines produced an effect which was additive to previously administered maximally effective doses of subcutaneous epinephrine. In 3 of 6 studies there was an additional response to subcutaneous epinephrine after a maximally effective dose of sympathicoa-aerosol. In one additional study there was no bronchodilation with the initial aerosol, but a significant response occurred with subsequently administered epinephrine subcutaneously. These findings are evidence that the mode of action of sympathicoamines differs depending on the way in which they are administered. Aerosolized drugs may have a more effective topical vasoconstricting effect, and the water vapor in the mist may have an effect in thinning mucus and permitting easier expectoration. Subcutaneously administered sympathicoamines may have a greater effect on vasoconstriction deep in the wall of the bronchus and on smooth muscle relaxation.

3. *The reproducability of the effects of sympathicoamines at different times in the same patient.* A variation of response to sympathicoamines was noted in 6 of the 10 patients studied on two or more occasions. This variability of response and the complete failure of a patient to respond to epinephrine in repeated doses by both aerosol and subcutaneous routes, which occurred in 17% of our studies, is not believed to be due to an

alteration of the pharmacologic effects of the drug (epinephrine fastness). Variation in the relative amounts of mucosal edema and congestion, bronchial inflammation, viscid secretion and bronchial muscle spasm which are the cause of airway obstruction in asthma is believed to be a plausible explanation of these phenomena.

Studies on Airway Resistance in Chronic Pulmonary Disease

By *William E. Ruth and Charles E. Andrews.* Medical Service, V.A. Hospital, Kansas City, Missouri and Department of Medicine, University of Kansas Medical School, Kansas City, Kansas.

An important ventilatory defect in bronchial asthma and pulmonary emphysema is a functional decrease in bronchiolar lumen with subsequent increase in resistance to airflow. The present study was undertaken to detect the presence and assess the magnitude of airway resistance (R) in chronic pulmonary diseases. The body plethysmograph offers a relatively simple and direct method for measurement of airway resistance without interference from the pressures necessary to overcome elastic and tissue viscance forces. A series of normal subjects and patients with chronic pulmonary diseases were studied.

In normal subjects (R) was found to be $1.25 \pm .21$ cm. H_2O/L./sec. Subjects with bronchial asthma who were free from signs or symptoms at the time of measurement had resistances ranging from 3.0 cm. H_2O/L./sec. to 4.8 cm. H_2O/L./sec. After nebulized Isuprel the mean decrease in (R) was 1.2 cm. H_2O/L./sec. Patients with pulmonary sarcoid had no increase in (R). Patients with clinical and laboratory evidence of pulmonary emphysema formed a heterogeneous group with (R) ranging from 2.0 to 11.0 cm. H_2O/L./sec. Of particular interest were patients with "bullous" emphysema in whom airway resistance was within the range of normal.

The following conclusions seem justified from the present study: (1) Airway resistance in asthmatics often is elevated at times when the patient is symptom-free and without physical findings. (2) "Bullous" emphysema appears to be a syndrome in which generalized increase in airway resistance is not found.

The Pulmonary Abnormalities in Myxedema

By *William R. Wilson and George N. Bedell.* Pulmonary Research Laboratory, Cardiovascular Laboratories, Department of Internal Medicine, College of Medicine, State University of Iowa, Iowa City.

The purpose of this paper is to report a study of lung function in 20 patients with myxedema. In these patients the mean basal metabolism rate was −22, the mean protein bound iodine 1.9 μg./100 ml., the mean I_{131} uptake at 24 hours 3% and the mean serum cholesterol 414 mg.%. Pulmonary function studies were done using standard methods previously reported from this laboratory.

Thirteen patients with myxedema and no clinical evidence of lung disease comprise Group I. They had normal arterial oxygen saturation and P_{co_2}. The mean values of pulmonary function tests were: vital capacity 87% PN (% PN equals % of predicted normal), residual volume 114% PN, total lung capacity 92% PN, maximal breathing capacity (MBC) 76% PN, maximal expiratory flow rate (MEFR) 220 L./min., maximal inspiratory flow rate (MIFR) 157 L./min., diffusing capacity ($D_{L_{co}}$) 17 ml./min./mm. Hg (66% PN) and hematocrit 34%.

Group II included 4 myxedematous patients with clinical or radiologic evidence of lung disease. One of these patients had roentgenologic abnormalities which improved as the euthyroid state was reached. Simultaneously, pulmonary function studies showed improvement in vital capacity and diffusing capacity. The other patients in this group had heart disease (2 patients) or emphysema (1 patient) in addition to myxedema.

Group III consists of 3 patients with myxedema and obesity (388, 318 and 277 pounds). These patients had alveolar hypoventilation manifested by arterial hypoxemia (79.6, 82.2 and 90.6%) and elevated arterial P_{co_2} (49, 54 and 60 mm. Hg). In these patients the mean values of pulmonary function tests were: vital capacity 69% PN, residual volume 41% PN, total lung capacity 57% PN, MBC 82% PN, MEFR 152 L./min., MIFR 88 L./min., $D_{L_{co}}$ 14 ml./min./mm. Hg (37% PN) and hematocrit 40%.

We conclude that myxedema produces mild reduction in lung volumes, reduced mechanical efficiency and definite reduction in the diffusing

capacity of the lungs. When myxedema was associated with obesity alveolar hypoventilation was found.

Cardiopulmonary Physiology in a Case of Pulmonary Alveolar Proteinosis

By *Thomas H. Snider and Benjamin M. Lewis.*
V. A. Hospital, Dearborn, Michigan and Department of Medicine, Wayne State University College of Medicine, Detroit.

Recently a new, chronic pulmonary disease, characterized by nonspecific respiratory symptoms and specific x-ray and pathologic findings, has been described. This disease was named pulmonary alveolar proteinosis because the alveoli were filled with a homogeneous, PAS-positive, proteinaceous material. The specific etiology of this condition has not as yet been determined.

Because of the relative rarity of this disease it was felt that an attempt should be made to delineate more carefully its pulmonary pathophysiology. To this end a patient was given a battery of pulmonary function tests including total and timed vital capacities, maximum breathing capacity and pulmonary compliance and diffusing capacity. He also underwent right heart catheterization.

The results of these studies placed this disease in the alveolar-capillary block group along with such diverse diseases as sarcoidosis, beryllium granulomatosis, alveolar cell carcinoma and scleroderma lung. The patient had a decreased total vital capacity with a normal timed (one second) vital capacity. The maximum breathing capacity was only slightly depressed. However, both pulmonary compliance and diffusing capacity, the latter measured by the rebreathing carbon monoxide technic, were markedly decreased. The only significant abnormal finding on cardiac catheterization was a slightly decreased arterial oxygen saturation at rest which further decreased on exercise rather than increased as in the normal.

Therefore we can conclude that the pathophysiologic changes in this condition constitute a "stiffening" of the lung parenchyma accompanied by a decrease in the flow of oxygen through the alveolar-capillary membrane. Thus another disease has been described which falls into the alveolar-capillary block syndrome.

TO READERS AND CONTRIBUTORS

An Experiment in Medical Journalism

The policy of Clinical Research is not only to give rapid publication to research abstracts, but also to provide a vehicle for kinds of articles which do not ordinarily find places in other publications.

These articles should be informed but brief statements of *views*, and they may cover a wide variety of professional matters. Their subject matter will range from purely scientific questions to discussions of research in general and of the environment in which research is carried on. Some of them will be solicited, but we hope that interested authors will submit such material of their own accord. The views expressed may well be somewhat partisan, and we expect that they will evoke counter-statements by workers who are not in agreement with them. A conscientious effort will be made to publish as many of these statements as space allows in early succeeding issues. Such contributions are of course subject to the customary editorial discretion.

Summary reviews of the usual type and original research communications (apart from abstracts) will not ordinarily be acceptable for the Journal, since numerous publication opportunities for such contributions already exist.

The aim of the policy is to provide a meeting place for medical minds, such that the membership of the American Federation for Clinical Research, and other interested persons, may benefit from the enormous amount of careful thought, unsupported by specific laboratory data, that is now being given to important professional issues by competent and conscientious workers. We would like our content to be often controversial without being contentious, and to point occasionally to worthwhile objectives without crusading.

The Editor and his associates solicit the advice and good will of readers of Clinical Research. The development of this experiment in medical journalism must depend on the willingness of competent persons to express themselves in print, and also on the willingness of readers of conviction to write in opposition to or in support of communications in the Journal. Such expression of opinion may take the form of "Letters to the Editor," and it is our hope that there will be an active Correspondence Section. Suggestions for changes in plan or for new activities will be welcomed.—*David T. Graham*

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